DOCTORAL THESIS

Responses in Vascular Function to Exercise in Women Aged 65-74 Years with Type 2 Diabetes

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RESPONSES IN VASCULAR FUNCTION TO EXERCISE IN WOMEN AGED 65-74 YEARS WITH TYPE 2 DIABETES

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by

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NAVIGATION OF THESIS

This thesis is made up of five chapters. Chapter One provides an overview of the literature with specific focus on the role of the microvasculature in the development of cardiovascular disease, particularly in women. Background information on the theory and methodology behind automated measurement of both retinal vessel calibre and fractal dimension is also described in detail. In Chapter One, the purpose and aims of this thesis are outlined.

Following Chapter One are three experimental studies, presented as three individual chapters (Chapters Two, Three, and Four). Each of the three experimental studies (Study One, Two, and Three) are distinct bodies of work that address the experimental aims of this thesis.

Chapter Five presents a discussion and conclusion section summarising the findings of the experimental studies in this thesis and provides information regarding the possible practical and/or clinical application of these findings.

ABSTRACT

The primary aim of this thesis is to examine changes in retinal microvascular structure and asymmetric dimethylarginine (ADMA) concentration in women aged 65-74 yr with type 2 diabetes, following twelve weeks of controlled and supervised exercise training at individual gas-exchange threshold (T_{ge}). In particular, this thesis examined the relationship between retinal vessel calibre/fractal analysis and measures of physiological functional capacity and endothelial function. Data from this thesis provide new knowledge on the effects of moderate intensity exercise on retinal microvascular morphology [retinal vessel calibre (RVC) and fractal dimension (D_f)] and ADMA concentration in women with type 2 diabetes. The same subjects were recruited for all studies.

Study One

The purpose of Study One was to investigate the relationship among retinal vessel calibre, fractal dimensions and physiological functional capacity in females aged 65-74 years with and without type 2 diabetes. Forty females (19 with type 2 diabetes and 21 without type 2 diabetes) underwent graded treadmill exercise testing to voluntary fatigue. Retinal photographs were obtained at a clinical eye examination from which retinal vascular calibre and fractal dimensions were quantified using a computer-based program (IRIS and IVAN) and summarized as the central retinal artery equivalent (CRAE) or central retinal vein equivalent (CRVE) and D_f. Subjects with type 2 diabetes had significantly lower peak oxygen uptake, peak heart rate, peak respiratory exchange ratio and time to exhaustion compared to age and gender-match subjects without type 2 diabetes. There were no significant differences between subjects with or without type 2 diabetes in CRAE (with diabetes 153.3±3.8 μm vs. without diabetes 154.7±2.7 μm, $p = 0.760$), CRVE (with diabetes 220.2±4.4 μm vs. without diabetes 230.8±4.9 μm, $p = 0.121$), or fractal dimension (with diabetes 1.45±0.004 vs. without diabetes 1.45±0.004, $p = 0.595$). In this sample of women aged 65-74 years, retinal vascular complexity (branching pattern) assessed as D_f was found to be significantly correlated ($r = 0.48$, $p = 0.04$) with time to exhaustion in individuals with type 2 diabetes. These findings provide the first evidence of a significant association between measures of physiological

functional capacity and retinal branching patterns in individuals with type 2 diabetes, which is considered a key parameter for the efficiency of microcirculation.

Study Two

While exercise training has been prescribed as a preventive and therapeutic intervention for cardiovascular disease in individuals with type 2 diabetes, the effects of exercise training on the retinal microvascular responses are not well described. Study Two investigated the effect of twelve weeks of supervised walking exercise on retinal vessel calibre and fractal dimension - markers of early microvascular complications - in women aged 65-74 years with type 2 diabetes. Fifteen women completed twelve weeks of supervised walking (120 minutes per week) at an intensity equivalent to their individual T_{ge}. Retinal photographs were taken and microvascular responses to exercise (via maximal exercise tests) were assessed before and after a 6-week intervention-free control period, and again after 6 and 12-weeks of exercise training. Twelve weeks of exercise training at T_{ge} resulted in significant increases in time to exhaustion ($p < 0.001$), peak oxygen uptake (VO_2peak) ($p = 0.016$), VO_2peak relative to body mass ($p = 0.026$), respiratory exchange ratio ($p = 0.040$), VO_2 at T_{ge} ($p = 0.030$), heart rate at T_{ge} ($p = 0.033$) as well as significant reductions in systolic ($p = 0.014$) and diastolic ($p = 0.032$) blood pressure. However, no significant changes in mean retinal vessel calibre or retinal fractal dimension were found after six or twelve weeks of exercise training. We could not document any significant changes in either RVC or D_f after twelve weeks of moderate-intensity, walking exercise in this sample of older women with type 2 diabetes. This contrasts with other studies showing that mild physical activity is associated with less adverse retinal microvascular signs.

Study Three

Basal plasma concentration of ADMA, an endogenous, competitive inhibitor of nitric oxide synthase, is elevated in patients with type 2 diabetes. ADMA may contribute to the endothelial dysfunction and associated vascular complications observed in individuals with type 2 diabetes. The purpose of Study Three was to investigate the effect of twelve weeks (120 minutes per week) of supervised walking exercise on plasma ADMA concentration in women aged 65-74 years with type 2 diabetes. Fourteen women (aged 69 ± 3 yrs) with uncomplicated type 2 diabetes, completed twelve weeks of supervised

walking at an intensity equivalent to their individual T_{ge}. Blood was sampled for ADMA concentration before and after a 6-week intervention-free control period, and again after six and twelve weeks of exercise training. Plasma ADMA concentration was found to be significantly lower after twelve weeks of exercise training when compared with baseline (wk 0) measurements. These results were accompanied by significant increases in time to exhaustion, relative and absolute VO_2peak, and VO_2 at T_{ge}. Regular, moderate-intensity exercise decreases circulating ADMA concentrations in older women with type 2 diabetes. These results suggest that ADMA may play a role in the training-induced reduction in cardiovascular disease risk seen with exercise training in individuals with type 2 diabetes.

Conclusion

The findings presented in this thesis support the use of regular, moderate-intensity exercise as an effective intervention for the management of type 2 diabetes in older women aged 65-74 years.

DECLARATION

This thesis is submitted to Bond University in total fulfilment of the requirements of the degree of Doctor of Philosophy. This thesis represents my own original work towards this research degree and contains no material which has been previously submitted for a degree or diploma at this University of any other institution, except where due acknowledgement is made.

Kevin Serre

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LIST OF PUBLICATIONS

The following publications are listed in support of this thesis;

Accepted for publication:

Serre, K., and Sasongko M.B. Modifiable Lifestyle and Environmental Risk Factors Affecting the Retinal Microcirculation. Microcirculation 19 (1), 29-36, 2012.

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Serre, K., Simmonds, M., Sabapathy, S., Minahan, C., Steele, M., Kawasaki, R., Wang, J.J., Wong, T., Mitchell, P., and Gass, G.C. Retinal Microvascular Responses to Exercise in Women aged 65-74 years with Type 2 Diabetes. Submitted for publication (2012).

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ABBREVIATED TERMS

ACEI	angiotensin-converting enzyme inhibitor
ADMA	asymmetric dimethylarginine
ARB	angiotensin-receptor blocker
ARIC	atherosclerosis risk in communities
AVR	arteriole-to-venule ratio
BDES	beaver dam eye study
BMES	blue mountain eye study
BMI	body mass index
BP	blood pressure
CHD	coronary heart disease
CKD	chronic kidney disease
CRAE	central retinal artery equivalent
CRVE	central retinal vein equivalent
CVD	cardiovascular disease
D_f	fractal dimension
DDAH-1	dimethylarginine dimethylaminohydrolase 1
EDTA	ethylenediaminetetraacetic acid
FEV_1	forced expiratory volume in 1 second
FPG	fasting plasma glucose concentration
FVC	forced vital capacity
GC	guanylate cyclase
GI	glycaemic index
GLUT-4	glucose transporter protein
GMP	guanosine monophosphate
GP	general practitioner
HbA1c	glycosylated haemoglobin
HDL	high-density lipoprotein
HRR	heart rate reserve
IFG	impaired fasting glucose

IGT	impaired glucose tolerance
IRIS	international retinal imaging software
IVAN	computer-based retinal grading program
LDL	low-density lipoprotein
MAP	mean arterial pressure
MESA	multi-ethnic study of atherosclerosis
NHPA	national health priority areas
NO	nitric oxide
NOS	nitric oxide synthase
OGTT	oral glucose tolerance test
PAR-Q	physical activity readiness questionnaire
PFC	physiological functional capacity
$PM_{2.5}$	fine particulate matter
RetVIC	retinal vascular imaging centre
RER	respiratory exchange ratio
RVC	retinal vessel calibre
SBP	systolic blood pressure
SCORM	singapore cohort study of the risk factors for myopia
TE	time to exhaustion
T_{ge}	gas-exchange threshold
TV	television
V_E BTPS	expired minute ventilation
VCO_2	carbon dioxide output
VO_2	oxygen uptake
VO_2peak	peak oxygen uptake
Wk	week
Yr	year

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CHAPTER 1 - *Introduction*

An Overview of the Literature and Statement of Purpose

An Overview of the Literature and Statement of Purpose

1.1 Ageing, Exercise and Women's Health

During 1991-2001, the female population in Australia, aged 65-74 yr, increased by approximately 8% to 682,000 and is expected to increase to 1.28 million by 2021 (Australian Institute of Health and Welfare 2006). The increasing number and life expectancy of older women in Australia poses a significant challenge and financial burden to our health-care system. Preventative and health promotion strategies must be developed for this group of Australians if chronic disease and disability are to be contained.

Ageing is associated with a decline in physiological functional capacity (PFC) that impairs health, well-being and independent living (Singh 2002). However, the rate of decline in PFC is variable, with some individuals showing greater rate of decline than others. Regular exercise is one factor that may contribute to the variability in PFC observed with ageing (Tanaka and Seals 2003). There is strong evidence suggesting that regular exercise may slow the rate of decline in PFC (Pollock et al. 1987; Tanaka and Seals 2003). Asmussen et al. (Asmussen et al. 1975) studied a group of 65 yr old, chronically active physical-education teachers and found that, unlike the control group, there was no deterioration in their PFC over a 12-yr period. Similar findings have been reported by others (Fries 1996), strengthening the "exercise hypothesis" that regular exercise can slow the rate of decline in PFC. Indeed, Fries (Fries 1980) went as far as to say; "in the absence of cigarette smoking, participation in regular endurance exercise can postpone the onset of chronic disease symptoms and manifestations." Evidence strongly suggests that the deleterious course of chronic diseases typically associated with ageing such as hypertension (Stewart 2002), type 2 diabetes (Orozco et al. 2008), osteoporosis (Kohrt et al. 1998), coronary artery disease (Mengelkoch et al. 1997) and the decline in cognitive function (Colcombe and Kramer 2003) can be positively modified by regular exercise. A recurring theme throughout the reviews (Colcombe and Kramer 2003; Kohrt et al. 1998; Mengelkoch et al. 1997; Orozco et al. 2008; Stewart 2002) was the importance to establish "the optimum exercise prescription" to achieve beneficial adaptations.

The direct economic costs of **physical inactivity** on the Australian Health Care system are estimated to be approximately $1.5 billion per year (Econtech 2007). Approximately 75% of older females are either **physically inactive** or exercise at a level insufficient to maintain or promote health benefits, a pattern that has remained essentially unchanged since 1989 (American College of Sports Medicine 2006). The most common medical problems managed by General Practitioners (GPs) for this age group involve circulatory (36%), musculoskeletal (23%) and respiratory (20%) complications (American College of Sports Medicine 2006). Evidence is accumulating that exercise is cardiorespiratory protective (Bassuk and Manson 2003; Powers et al. 2004), vascular protective (Hambrecht et al. 1998), musculoskeletal protective (Asikainen et al. 2004), and neuroprotective (Mattson et al. 2002). Regular exercise can compress morbidity (Fries 1980, 2003; Winett et al. 2003) and significantly reduce the disability index (↓ 300%) in women (Bruce and Fries 2003). Fries (Fries 1996) reported that physically active middle-aged and older women showed no significant increase in their disability index over an 8-yr period while they maintained their running history. Physically **inactive** women over the same 8-yr period increased their level of disability three fold. For older women, moderate exercise such as walking represents a potent intervention with multi-focal systemic benefits. If exercise is to have health benefits, the appropriate dose of exercise must be known to achieve those benefits without incurring an increased risk of injury or attrition.

We do not know the interaction effects of the key exercise prescription principles (intensity, duration, frequency, mode and total work) that will maximize health benefits desired by GPs for this group of Australians. If exercise e.g., walking, is to be prescribed by GPs and other credentialed health professionals for their older female patients, health professionals need to know the effect size, the response time, and threshold point for select outcome measures if the desired health benefits are to be attained. While the benefits of regular exercise in this population are described (for review see Thomas et al. 2006), key exercise prescription principles remain unclear. The proposed study will examine the effects of exercise, set at an intensity equivalent to individual gas exchange threshold, on select outcome measures in women aged 65-74 years old with and without type 2 diabetes.

1.2 Type 2 Diabetes Mellitus

Type 2 diabetes is a metabolic disorder characterized by hyperglycaemia, insulin resistance, beta-cell dysfunction, and abnormal glucose, lipid and protein metabolism, resulting from defective insulin production, insulin action, or both (Thomas et al. 2006). Type 2 diabetes occurs mostly in persons over the age of 40 yr and accounts for about 90% to 95% of all diagnosed cases of diabetes (Australian Institute of Health and Welfare 2008) (**Figure 1**).

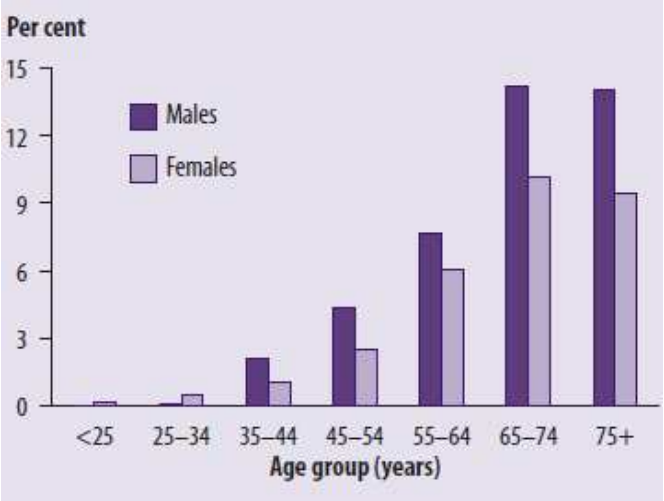


Figure 1. Age-specific prevalence of diagnosed type 2 diabetes, 2004-2005. (Australian Institute of Health and Welfare 2008)

Traditionally, diagnosis of type 2 diabetes was based on either plasma glucose concentration or an oral glucose tolerance test. More recently, the American Diabetes Association has expanded the criteria for the diagnosis of diabetes to: A. glycosylated haemoglobin (HBA_{1c}) value ≥ 6.5%; B. fasting plasma glucose concentration (FPG) ≥ 126 mg/dL (7.0 mmol/L) (following no caloric intake for at least 8 h); C. 2-h plasma glucose ≥ 200 mg/dL (11.1 mmol/L) during a 75-g oral glucose tolerance test (OGTT); or D. a patient showing classic symptoms of hyperglycaemia or hyperglycaemic crisis and a random plasma glucose ≥ 200 mg/dL (11.1 mmol/L) (American Diabetes Association 2012b). Criteria for identifying individuals with increased risk of developing future diabetes, known as pre-diabetes (American Diabetes Association 2012a), have also been established. According to the American Diabetes Association, individuals with: A. FPG 100mg/dL (5.6 mmol/L) to 125 mg/dL (6.9 mmol/L) (impaired fasting glucose); and/or B. 2-h plasma glucose between 140 mg/dL (7.8 mmol/L) and 199 mg/dL (11.0 mmol/L)

during a 75-g OGTT [impaired glucose tolerance (IGT)]; and/or C. HBA_{1c} 5.7-6.4% should be considered to be at high risk for developing diabetes (American Diabetes Association 2012b).

Due to the progressive nature, type 2 diabetes is frequently undiagnosed until overt complications appear. In Australia, population studies have estimated that there may be as many as one case of undiagnosed diabetes for every known case (Dunstan et al. 2002). Given that diabetic trials have proven lifestyle interventions to be effective in significantly decreasing the rate of onset of diabetes (Knowler et al. 2002; Tuomilehto et al. 2001), early identification of diabetes risk is essential. Due to the significant pre-symptomatic phase often associated with type 2 diabetes, the American Diabetes Association (American Diabetes Association 2012b) has established criteria for testing for diabetes in asymptomatic adult individuals. The American Diabetes Association (American Diabetes Association 2012b) suggests that testing should be considered for all adults who are:

1. Overweight [body mass index (BMI) ≥ 25 kg/m^2] and have one or more additional risk factors:
    - physical inactivity
    - first degree relative with diabetes
    - high-risk race/ethnicity
    - women who delivered a baby weighing > 9lb or who were diagnosed with Gestational Diabetes
    - hypertension (blood pressure ≥ 140/90 mmHg)
    - HDL cholesterol level < 35 mg/dL and/or a triglyceride level > 250 mg/dL
    - women with polycystic ovary syndrome
    - HbA1c ≥ 5.7%, IGT, or impaired fasting glucose (IFG) on previous testing
    - history of cardiovascular disease (CVD)
2. In the absence of the above criteria, testing should begin at age 45 years
3. If results are normal, testing should be repeated every 3 years

(American Diabetes Association 2012b)

Long-term type 2 diabetes is associated with with macrovascular (coronary, peripheral vascular), microvascular (retinal, renal), and neuropathic (autonomic, peripheral)

complications. Type 2 diabetes is associated with older age, obesity, family history of diabetes, history of gestational diabetes, impaired glucose metabolism, physical inactivity, and race/ethnicity. Diabetes is recognized as one of Australia's top nine National Health Priority Areas (NHPAs) (Australian Institute of Health and Welfare 2010), with an estimated 5.2% of all deaths directly due to diabetes (Roglic et al. 2005). With approximately 90000 Australians diagnosed every year with type 2 diabetes (Dunstan et al. 2002), effective prevention strategies and treatment options has become a high health priority. The Australian Institute of Health and Welfare estimates the direct health-care expenditure on diabetes to be $907 million, nearly 2% of the allocated recurrent health expenditure (Australian Institute of Health and Welfare 2008). Obesity and **physical inactivity** represent two major modifiable risks factors for type 2 diabetes in adults and represent key risk factor foci of prevention and treatment. Recent statistics suggest that 51% of Australians aged 15 years or older are overweight or obese and 70% did insufficient physical activity to promote or maintain health (Australian Institute of Health and Welfare 2008). Of all the complications associated with type 2 diabetes, cardiovascular disease is the leading cause of death in individuals with the disease (Australian Institute of Health and Welfare 2008).

1.3 Type 2 Diabetes Mellitus and Atherosclerosis

"A man is as old as his arteries"
- Thomas Sydenham (1624-1689)

Type 2 diabetes magnifies the risk of CVD morbidity and mortality in older individuals (Resnick et al. 2001). Numerous factors have been identified that increase the risk of developing CVD, including – but not limited to – heredity, **physical inactivity**, smoking, impaired fasting glucose, hypertension, dyslipidaemia, and age (Roger et al. 2012). Cardiovascular disease (central and peripheral vascular disease) is also a major complication associated with type 2 diabetes. Since CVD is the principal complication in type 2 diabetes and a major cause of death and disability in older individuals (Howard and Magee 2000), early detection and prevention of type 2 diabetes is important to the individual and to the health system. Estimates are that up to 75% of individuals with type 2 diabetes will die of CVD (de Jager et al. 2006). Peripheral vascular dysfunction is well

documented in sedentary older individuals and in individuals with type 2 diabetes (Bernard et al. 2005; Zeiher et al. 1991). An integral part of that vascular dysfunction is endothelial function (Wang et al. 2006a). Individuals with clinically evident cardiovascular disease (or cardiovascular risk factors) demonstrate an impaired ability of the vascular endothelium to respond to appropriate stimuli. In these individuals, evidence of endothelial dysfunction predicts adverse outcomes (Halcox et al. 2002; Perticone et al. 2001). While patients with acute coronary syndrome demonstrated endothelial dysfunction, improved endothelial function was achieved by repeated bouts of exercise and associated with improved health outcomes (Schachinger et al. 2000). Understanding the link between type 2 diabetes and CVD is an important step in establishing effective medical therapy.

1.4 Diabetes and Endothelial Function

The epidemiology and pathophysiology of changes in blood vasculature with diabetes remains uncertain. What is known is that clinical conditions that accompany diabetes (e.g. hyperglycaemia, hyperlipidaemia, insulin resistance) cause arterial dysfunction and render arteries susceptible to atherosclerosis (for review see Beckman et al. 2002). **Figure 2** outlines the cascade of consequences of diabetes mellitus on endothelial function, which ultimately leads to atherosclerosis.

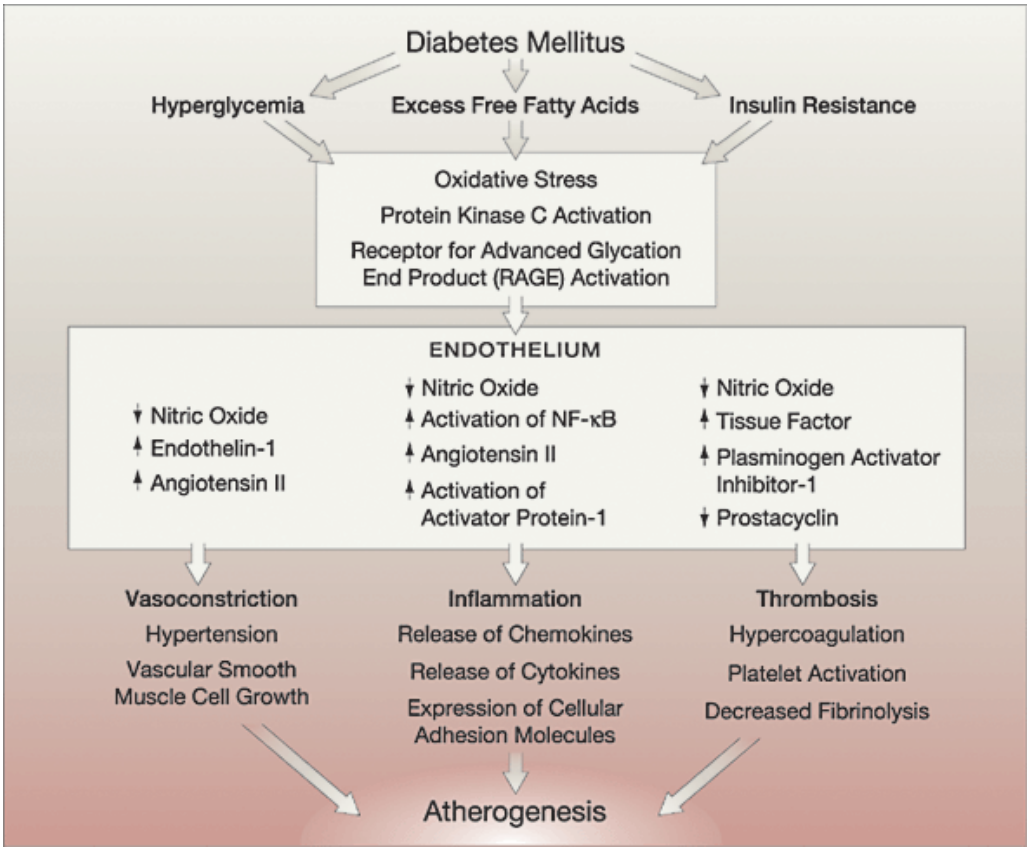


Figure 2. Endothelial dysfunction in diabetes. (Beckman et al. 2002).

The endothelium is a thin layer of cells that line the interior surface of blood vessels and form an interface between circulating blood and vessel walls (for review see Davignon and Ganz 2004). The endothelium provides a metabolically active interface between blood and vessel walls that allows for nutrient delivery, coagulation and thrombosis (Cines et al. 1998) and synthesizes bioactive substances [e.g. nitric oxide (NO), endothelin-1] that regulate vasculature function and structure. Diabetes impairs nitric oxide-mediated vasodilation as well as increases the production of vasoconstrictors (endothelin-1) (Beckman et al. 2002), which leads to atherosclerotic changes in the vasculature. Together, changes associated with diabetes (i.e. hyperglycaemia, hyperlipidaemia, and insulin resistance) impair endothelial function, alter vasoconstriction, increase inflammation, and promote thrombosis, overall enhancing the initiation of atherogenesis and producing clinical signs/symptoms of atherosclerosis.

1.5 Effects of Exercise on Endothelial Function

The role of endothelial dysfunction in the development of atherosclerosis is well established, in particular, plaque formation, fibrinolysis and the regulation of arterial pressure (for review see Tousoulis et al. 2010). The effects of regular exercise on improving endothelial function have been well documented (for review see Ghisi et al. 2011). Evidence suggests that exercise-induced activation of the NO pathway is an important mechanism in the vascular improvements associated with repeated bouts of exercise (for review see Ghisi et al. 2011). Endothelial-dependent vasodilation has been shown to increase with regular exercise and is principally mediated by NO (Casillas et al. 2007). Repeated bouts of exercise stimulate the expression of molecular factors involved in NO production (Dimmeler and Zeiher 2003; Fukai et al. 2000). Figure 3 shows the proposed adaptation of peripheral and coronary vasculature to exercise as proposed by Maiorana et al (Maiorana et al. 2003). **Figure 3** (Panel A)**,** shows an untrained vessel in which baseline endothelial release of NO (shown in yellow), which diffuses to smooth muscle, activates guanylate cyclase (GC) leading to production of cyclic guanosine monophosphate (GMP). Cyclic GMP leads to calcium channel opening and causes smooth muscle relaxation and vasodilation of the vessel. **Figure 3** (Panel B) shows a vessel after several weeks of exercise training. The repeat bouts of exercise are suggested to increase the shear stress along the vessel wall as a result of pusatile blood flow, which stimulates increased endothelial NO production and the consequent vasodilation (Maiorana et al. 2003). Up-regulation of endothelial nitric oxide synthase (NOS) expression occurs as means of buffering increased shear stress. **Figure 3** (Panel C) depicts structural adaptations (increased vessel calibre) that occur following long-term exercise training. Chronic increases in vessel calibre are seen due to NO-mediated changes in smooth muscle cells and NO function returns towards baseline levels (Maiorana et al. 2003). Exercise training has been established to positively modify flow-dependent, endothelium-mediated vasodilation in patients with cardiovascular disease (Hambrecht et al. 1998; Hambrecht et al. 2003; Hornig et al. 1996) and in patients with type 2 diabetes mellitus (Maiorana et al. 2001). While the effects of exercise on endothelial dysfunction in patients with diabetes have been examined, an exercise dose-

response relationship remains unknown, as does the role of asymmetric dimethylarginine (ADMA), a NOS inhibitor.

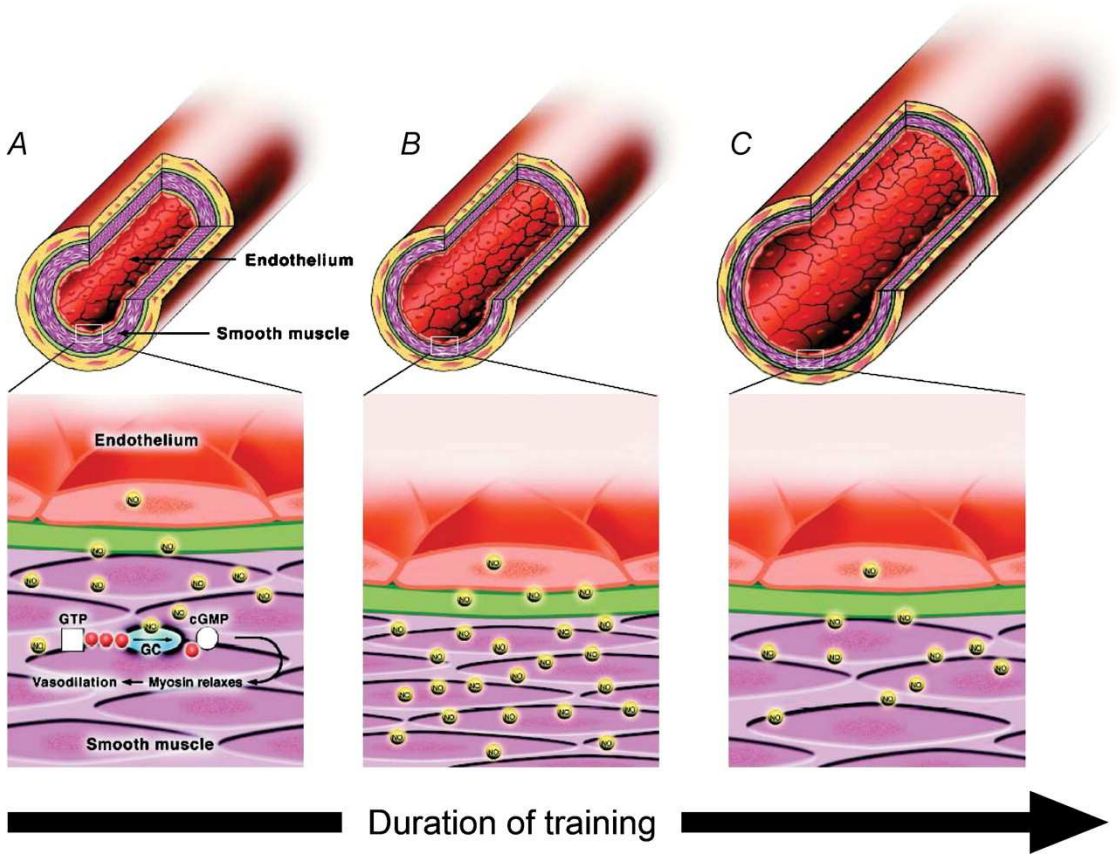


Figure 3. Hypothesised response of arteries to increased flow and shear stress following varying duration of exercise training. (Maiorana et al. 2003).

1.6 Type 2 Diabetes and CVD: Gender Differences

Type 2 diabetes mellitus is associated with an increased risk of CVD, cerebrovascular disease, and peripheral vascular disease (Kanaya et al. 2002). While type 2 diabetes increases the risk of CVD in both men and women, the increase in risk of CVD is more marked in women than in men (Avogaro et al. 2007; Juutilainen et al. 2004; Kanaya et al. 2002). Women with type 2 diabetes, compared to age-matched women without diabetes, have a 5-to-7 fold higher rate of CVD death (Steinberg et al. 2000), producing event rates similar to those seen in similarly aged men with type 2 diabetes. While the impact of gender on risk of CVD in individuals with type 2 diabetes is well established (Avogaro et al. 2007; Juutilainen et al. 2004; Kanaya et al. 2002), the mechanisms behind these gender differences remain uncertain. In 2000, Steinberg (Steinberg et al. 2000) and

colleagues examined gender differences in endothelial function as a possible explanation for the elimination of the usual female advantage for CVD mortality. They found that women exhibit higher rates of NO production than men and the gender differences in NO production is abrogated in type 2 diabetes, lessening the cardioprotective action of NO. In a 20-year follow up study, Hu et al (Hu et al. 2001) found that type 2 diabetes was associated with significant increases in the risk of total mortality and CVD death among women, increasing risk of fatal CVD to nearly that of conferred by prior CVD. If the gender differences in CVD risk to diabetics are to be better understood, further studies are required to examine the effects of diabetes on NO action, along with NO synthase inhibitors, including ADMA.

1.7 Type 2 Diabetes Mellitus and Exercise

Together with medication and diet intervention, exercise has been well established as an effective intervention in the management of type 2 diabetes (Marwick et al. 2009). To date, several studies (for review see Thomas et al. 2006) have investigated the effect of repeated bouts of aerobic exercise on type 2 diabetes in various populations. Significant decreases in glycaemia (Lee et al. 2005), increases in insulin sensitivity, insulin secretion and/or decreases in HbA1c concentration (Boule et al. 2001; Hulver et al. 2002; Mourier et al. 1997; Thomas et al. 2006; Willey and Singh 2003), reduction in BMI, total fat mass, sum of skin-fold measurements, waist and/or waist-to-hip ratio (Christ-Roberts et al. 2004; Hulver et al. 2002; Lee et al. 2005; Ross et al. 2004), improvements in cardiovascular risk profile (increase of high-density lipoprotein/low-density lipoprotein (HDL/LDL) ratio, normalization of blood lipid profile and a reduction of systolic and diastolic blood pressure) (Banz et al. 2003) have all been documented benefits of aerobic exercise in individuals with type 2 diabetes.

Skeletal muscle consumes between 70% to 90% of glucose transported in blood (Lambers et al. 2008). Single bouts of exercise have been reported to increase active muscles' insulin sensitivity, while regular exercise has been shown to cause long-term improvements in glycaemic control, both providing type 2 diabetics with lowered insulin requirements (Lambers et al. 2008). Improved insulin sensitivity results from the

translocation of glucose transporter protein (GLUT-4) from the endoplasmic reticulum to the cell surface, increased total quantity of GLUT-4, and an increase in glycogen synthase activity and subsequent glucose storage as glycogen (Sigal et al. 2006). The improved insulin sensitivity and the attending physiological effects resulting from regular bouts of exercise are important in the management of type 2 diabetes.

1.8 Asymmetric Dimethylarginine

1.8.1 Nitric Oxide and Vascular Homeostasis

Endothelium-derived NO is an endogenous vasodilator, important to flow-mediated vasodilation and a critical modulator of blood flow and blood pressure (Pohl et al. 1986; Rees et al. 1989). Loss of NO activity has been shown to accelerate the development of vascular lesions, occurring early in the course of vascular disease (Celermajer et al. 1994). Evidence has shown that a reduction in endogenous NO may play an important role in increasing vascular resistance and the initiation and development of atherosclerosis and therefore may be predictive of vascular events (Cooke 2000). Endothelial dysfunction, via changes in the NO pathway are caused primarily by reductions in: (i) NO half-life, (ii) NO sensitivity, (iii) NOS expression, and (iv) NOS activity (Cooke 2000). There is accumulating evidence that endogenous inhibition of NOS activity may be responsible for much of the endothelial dysfunction seen in individuals with vascular disease and cardiovascular risk factors (Cooke 2000). The most predominant endogenous inhibitor NOS activity is ADMA.

1.8.2 Asymmetric Dimethylarginine, Nitric Oxide Synthase, and Endothelial Dysfunction

ADMA is an endogenous competitive inhibitor of NOS (Lin et al. 2002). ADMA has been implicated in the progression of endothelial dysfunction, limiting the synthesis of vasoprotective NO and accelerating atherosclerosis (Cooke 2005). Plasma levels of ADMA have been found to be increased in conditions associated with atherosclerosis and with cardiovascular risk factors, including age, hypertension, hypercholesterolemia, hypertriglyceridemia, hyperhomocysteinaemia, insulin resistance and diabetes (Abbasi et

al. 2001; Boger et al. 1998; Miyazaki et al. 1999; Stuhlinger et al. 2002). Current evidence suggests that ADMA levels may be predictive of cardiovascular events and may be an independent risk factor for central and peripheral vascular disease (Cooke 2005). By blocking NO synthesis, ADMA promotes vasoconstriction, along with inflammatory, oxidative, proliferative and adhesive processes crucial in atherogenesis (Richter et al. 2005). Small changes in ADMA concentrations have been found to significantly alter vascular NO production, vascular tone, and systemic vascular resistance (Boger 2003). The pathophysiological roles of elevated ADMA levels, resulting from its inhibition of endothelial NO formation can be seen in **Figure 4**.

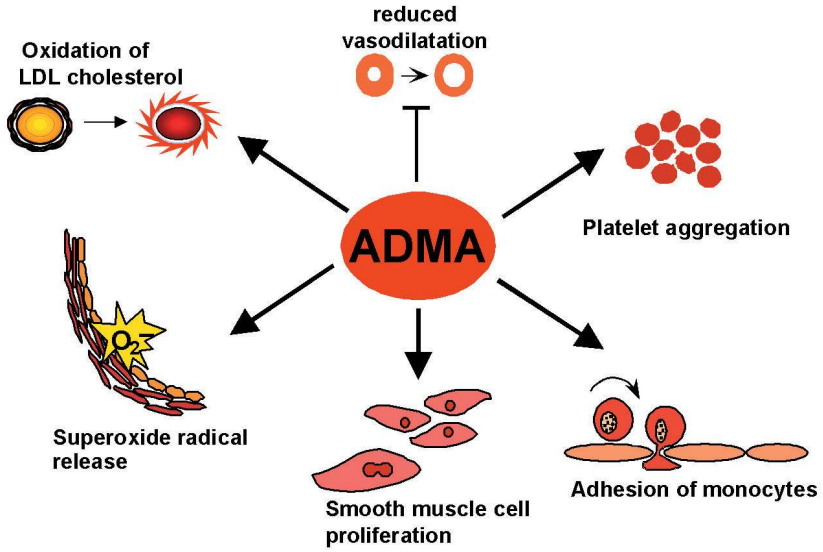


Figure 4. Pathophysiological consequences of elevated ADMA concentrations. (Boger 2004).

1.8.3 Asymmetric Dimethylarginine, Type 2 Diabetes Mellitus, and Vascular Disease

While CVD is a major cause of morbidity and mortality in individuals with type 2 diabetes (Astrup 2011), the elevated incidence of CVD cannot be fully explained by the presence of conventional risk factors (Hayden and Reaven 2000). Recent studies have presented compelling evidence for the association between elevated ADMA concentration and atherosclerosis (Abbasi et al. 2001; Cooke 2005). Conditions associated with atherosclerosis, including type 2 diabetes mellitus, are associated with increased plasma ADMA concentration (Abbasi et al. 2001; Cooke 2005). In 2001,

Abbasi and colleagues (Abbasi et al. 2001) examined plasma ADMA concentrations in normal volunteers and in individuals with type 2 diabetes. Significantly higher ADMA concentrations were found in individuals with type 2 diabetes matched for age, gender distribution, body mass index, and total and low-density lipoprotein cholesterol concentrations (Abbasi et al. 2001) (**Figure 5**). The increased incidence of cardiovascular disease associated with diabetes cannot be explained by traditional risk factors, leading to suggestions that ADMA's role in NO synthase inhibition is a possible factor in the accelerated atherogenesis associated with type 2 diabetes (Abbasi et al. 2001; Boger et al. 1998; Fard et al. 2000; Hayden and Reaven 2000; Lin et al. 2002).

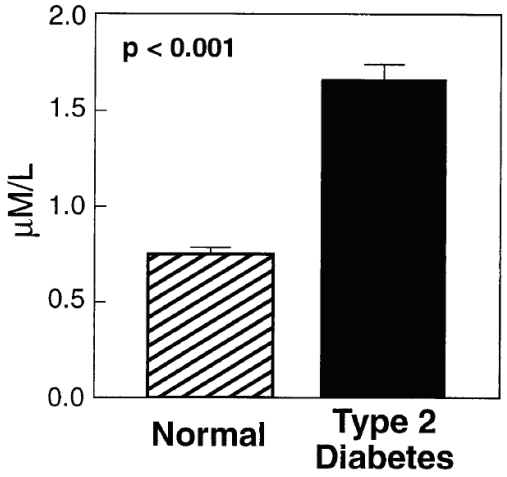


Figure 5. Comparison of plasma ADMA concentration (μM/L) in normal subjects and patients with type 2 diabetes. (Abbasi et al. 2001).

1.8.4 Asymmetric Dimethylarginine and Exercise

To date, no studies have investigated the effects of repeated bouts of exercise on ADMA concentration in individuals with type 2 diabetes mellitus. However, in persons at risk of CVD, exercise has been shown to reduce ADMA concentration (Richter et al. 2005), with those persons showing significant down-regulation of circulating ADMA concentration after twelve weeks of aerobic exercise at moderate intensity. These results suggest lifestyle modifications, such as regular exercise, may be a considered approach to reduce ADMA concentration. The effects of repeated bouts of exercise upon ADMA concentration may in part explain previously unknown mechanisms for observed

improvements in endothelial function following exercise training (Boger et al. 1998). While the current literature on the effects of exercise on ADMA concentration is sparse, information on a possible dose-response relationship between decreases in plasma ADMA concentrations and exercise is non-existent. Another proposed marker of endothelial function is retinal microvascular morphology.

1.9 Retinal Vascular Calibre as a Mirror of Microvascular Changes

Retinal vasculature provides a direct and non-invasive view of the condition of microcirculation (Wong et al. 2001b). Retinal arterioles and venules share many anatomical and physiological characteristics with the cerebral, coronary, peripheral, and renal microcirculations (Klein et al. 2007). Studies have shown that changes in retinal vascular calibre (RVC) [i.e., retinal arteriolar narrowing, retinal venular widening and reduced ratio of arterioles/venules (AVR)] have been associated with CVD, including stroke (Wong et al. 2001a), coronary heart disease, hypertension and metabolic disorders (Wong et al. 2002c). Studies have also shown that retinal vascular changes are associated with heart failure (Wong et al. 2005a) and cardiovascular mortality (Klein et al. 2004) in individuals with type 2 diabetes. It has been suggested (Wong et al. 2002c) that the underlying basis for the association between retinal vessel calibre and vascular disease may be related to endothelial dysfunction. Particularly relevant to the proposed study is the well-documented relationship between RVC and CVD. The relationship between RVC and CVD has been reported to be stronger in women than in men (Duncan et al. 2002; Tedeschi-Reiner et al. 2005; Wong et al. 2002c), thereby raising the significance of the proposed study. The reasons for the stronger relationship between RVC and CVD in women are unclear. Studies have suggested that in women, endogenous oestrogen may play a cardioprotective role before menopause and with the onset of menopause, the reduction in oestrogen accelerates the rate of CVD post menopause (Hodis et al. 2001). The decline in plasma oestrogen concentration has been suggested as a significant contributory factor to the accelerated rate of CVD. Studies have also suggested that the stronger relationship between RVC and CVD in women is that microvascular disease may have a greater role in the pathogenesis of CVD in women than in men (Buchthal et al. 2000; Hasdai et al. 1998; Reis et al. 1999). While some clinicians have begun to

include retinal photography as a component of their clinical evaluation of women at risk of myocardial ischaemia and CVD (Shaw et al. 2004), other clinicians have stressed the need to confirm the categorical link between endothelial dysfunction and increased risk of CVD in postmenopausal women (Bressler 2003; Maguire 2003). Understanding the contribution of microvascular processes to the risk of CVD may prove important in establishing preventative and therapeutic interventions (Wong et al. 2002c), including a prescribed dose of regular exercise.

By using non-invasive measurement of retinal vessels (fundus photography and computerized analysis), the retinal vasculature may provide a useful surrogate marker of endothelial dysfunction and how selected doses of exercise might affect system-wide endothelial function. Changes in retinal vasculature (eg. retinal arteriolar narrowing, retinal venular widening and reduced AVR) may be used as markers of early pre-clinical stages of cardiovascular diseases and metabolic disorders (Liew et al. 2008c) (**Table 1**). A better understanding of the associations between retinal vasculature and markers of CVD and the interaction between them may allow for use of retinal microvascular calibre as a biomarker for sub-clinical disease and a surrogate marker for cardiovascular response to therapy. By examining stimuli that improve endothelial function, e.g. regular exercise, a better understanding of the mechanism(s) for the improvements in endothelial function with regular exercise may be proposed.

Table 1. Conditions associated with changes in central retinal artery equivalent (CRAE) and central retinal vein equivalent (CRVE).

CRAE	CRVE
Smaller CRAE is associated with: • Aging • Higher blood pressure (Current/Past) • Smaller optic disc • Obesity/BMI • Migraine • Hypertension • Stroke	Larger CRVE is associated with: • Obesity • BMI • Inflammatory marker • Diabetes • Metabolic Syndrome • Low HDL cholesterol • Diabetes • Obesity • Progression of retinopathy • CHD • Cardiovascular mortality
Larger CRAE is associated with: • Progression of retinopathy	

1.9.1 Assessment of Retinal Vessel Diameters – IVAN Software

IVAN is a system developed at the University of Wisconsin as a semi-automated means of measuring retinal vessel widths (CRAE/CRVE) from digital retinal images (**Figure 6**).

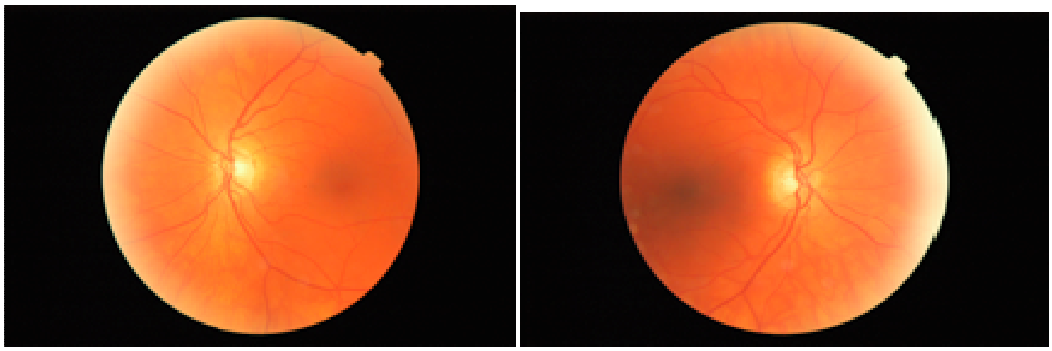


Figure 6. Sample of retinal photo taken from a patient for grading of retinal vessel calibre.

During analysis, IVAN software places an overlying grid centred on the optic disc, and proceeds to identify blood vessel types and measure widths of the identified blood vessels. Specifically, IVAN software measures internal vessel calibre or erythrocyte column width. Venules are denoted by the colour blue and arterioles are denoted by the

colour red. As seen in **Figure 7**, all retinal vessels passing through an area between 0.5 and 1.0 standard disc diameters from the optic disk (Zone B) are identified and measured. Trained graders are able to override any initial automated decisions or measurements, including grid placement, changing vessel type, deleting vessels or adding missed vessels.

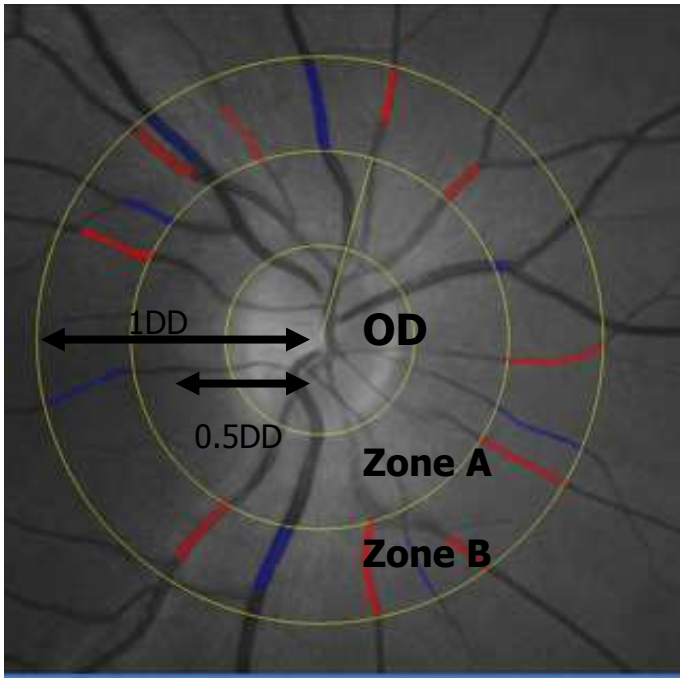


Figure 7. Retinal vessel diameters passing through an area between circles with a 0.5 and 1.0 standard disc diameters from the optic disc margin (**Zone B**).

Measurement results are calculated, using the six largest venules and six largest arterioles, as CRAE, CRVE, and AVR, providing estimated central retinal artery and vein diameter based on branch measurements. AVR provides a ratio between CRAE and CRVE measurements. **Figure 8** depicts two retinal photographs from two different individuals. One photograph (A) shows a retinal photograph with a low AVR [arterioles (white arrows) are smaller than venules (black arrows)] and a second photograph (B) shows a retinal photograph with a high AVR (arterioles are wider than venules).

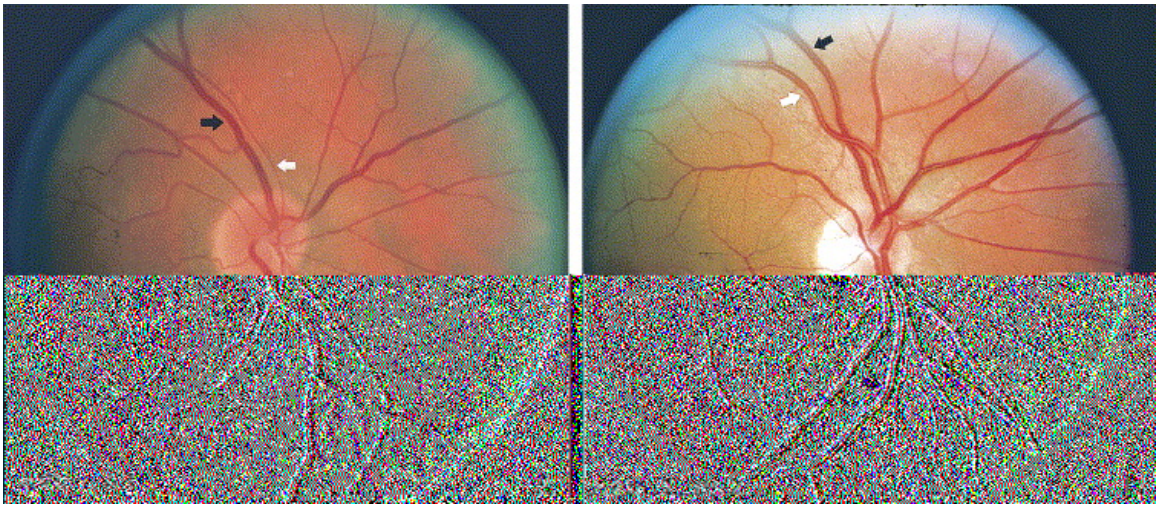


Figure 8. The AVR of two retinal photographs. **(A)** Low AVR and **(B)** High AVR. (Wong et al. 2004b).

1.10 Fractal Analysis of the Retinal Microvasculature

1.10.1 Fractal Geometry – Fractals in Biology

> *"I conceived and developed a new geometry of nature and implemented its use in a number of diverse fields. It describes many of the irregular and fragmented patterns around us, and leads to full-fledged theories, by identifying a family of shapes I call fractals."*
>
> — Benoit Mandelbrot, 1983

A fractal, first coined by Beniot Mandelbrot, is "a rough or fragmented geometric shape that can be split into parts, each of which is (at least approximately) a reduced-size copy of the whole", a property known as self-similarity (Mandelbrot 1983).

Fractals are often considered to be infinitely complex, with greater complexity revealed under magnification (**Figure 9**). As most physical systems are not easily described through Euclidian geometry, fractals have been used to describe, measure and predict complex natural structures (e.g. branching patterns of trees, coastlines, snowflakes, and lightning bolts).

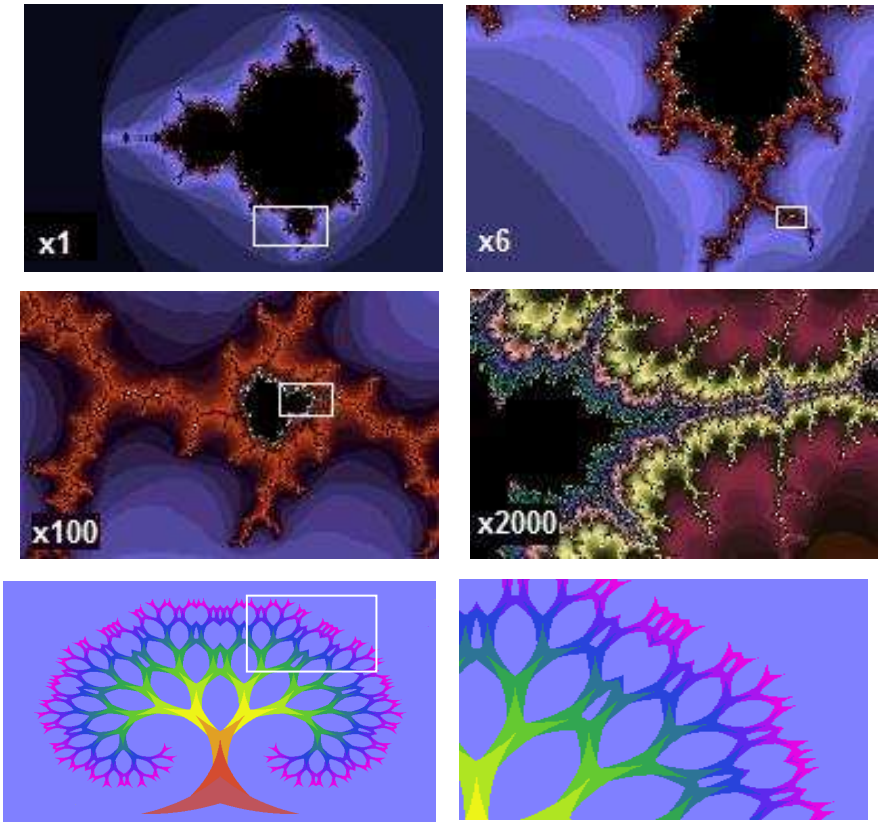


Figure 9. Example fractal images displaying self-similarity and increasing complexity under magnification. (Source: www.fractal.org).

The theory that fractal geometry may serve as a design principle underlying living organisms has been elegantly presented (Weibel 1991). The application of fractal analysis to biology and medicine has recently gained much interest. When applied to branching structures in tissues and organs, fractal analysis has allowed for discrimination between normal and abnormal or pathological structures (Masters 2004). Fractal analysis has been successfully used in treatment and tracking the progress of Parkinson's Disease (Sekine et al. 2004), Alzheimer's Disease (Nagao et al. 2001), and Multiple Sclerosis (Esteban et al. 2009). Fractal analysis has also been applied in coding region detection in DNA and measurement of space-filling properties of tumours and blood vessels (Cross 1997).

A long-standing problem in vascular pathophysiology is the characterization of blood vessel patterns. Atherosclerosis, cancers, infections, stroke, hypertension, diabetes, obesity, and Alzheimer's Disease are associated with changes in vascular morphology and organization, along with normal aging (Lorthois and Cassot 2010). By characterizing

vascular branching structures into fractal dimensions, changes in vascular complexity may become a useful indicator of early, pre-clinical vascular change. Research has shown that the retinal vasculature exhibits "self-similarity" and its vascular tree can be considered a fractal structure (Macgillivray et al. 2007; Mainster 1990; Masters 2004; Stosic and Stosic 2006), and can be quantified through analysis of digital retinal images.

1.10.2 Retinal Vascular Fractals

The application of fractal analysis to human retinal circulation was first introduced in 1989 (Family et al. 1989; Masters and Platt 1989). Using early techniques of calculating D_f, Masters and colleagues were able to estimate a retinal D_f of 1.7, which is consistent with a diffusion-limited growth process, the random growth process by which fractals are formed (Masters 2004).

1.10.3 Assessment of Retinal Fractal Dimensions – International Retinal Imaging Software

International Retinal Imaging Software, or IRIS, is a semi-automated grading program which aims to measure the D_f of retinal vasculature. By calculating the D_f for digital retinal photos, the branching patterns of the retinal vascular tree can be summarized as an estimate of the amount of "space" a fractal pattern occupies (**Figure 10**). The D_f provides a more global measure of retinal microvascular structure than CRAE, CRVE or AVR (as measured by IVAN software) by taking into account both retinal vessel calibre and vascular branching patterns (Liew et al. 2008b).

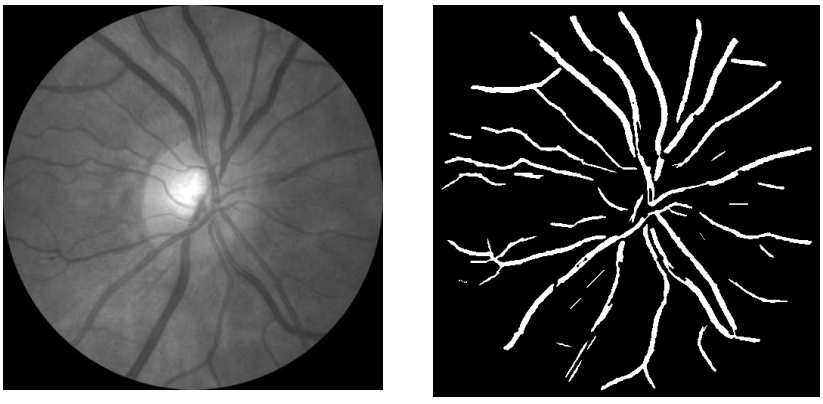


Figure 10. IRIS automated calculation of D_f.

1.10.4 Use of Retinal Vascular Fractals

While research into retinal vascular fractal analysis is still developing, a number of significant relationships have been found. Data from Liew *et al*. (Liew et al. 2008b) (**Figure 11**) shows that D_f exhibits a significant inverse relationship with systolic blood pressure (SBP), suggesting that D_f may be a measure of early changes in microvascular structure from elevated systolic blood pressure.

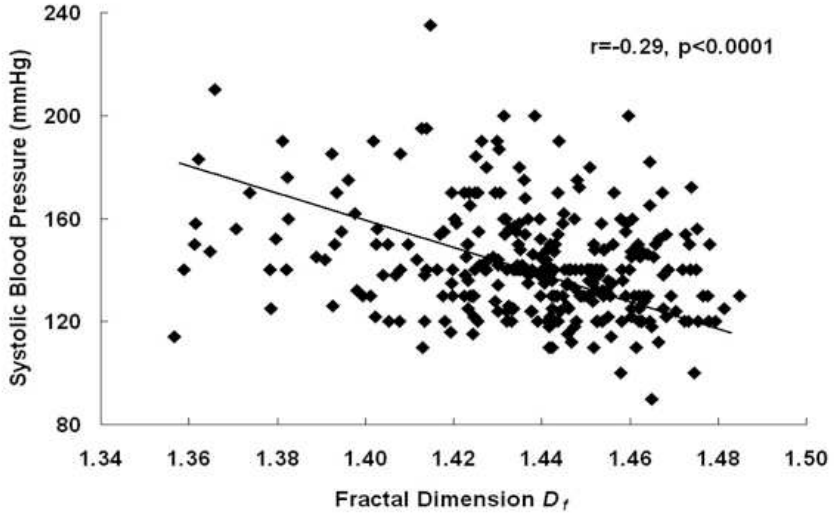
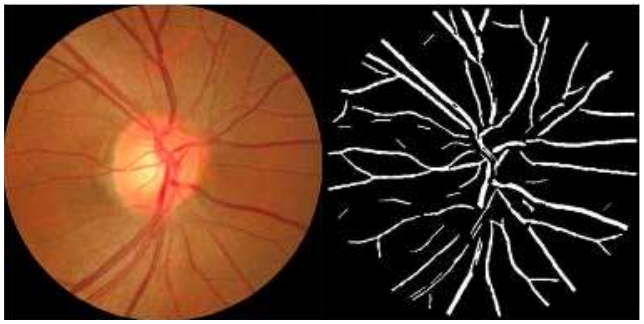
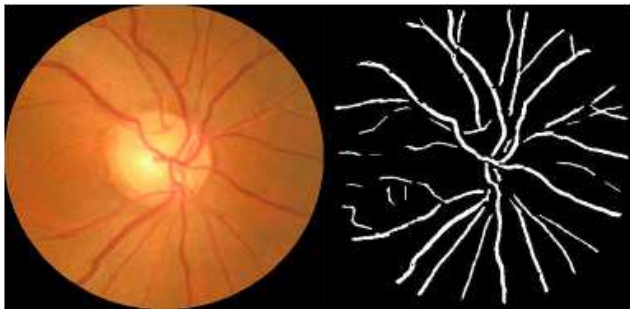


Figure 11. Fractal dimension and systolic blood pressure in 300 participants of Blue Mountain Eye Study. (Liew et al. 2008b).

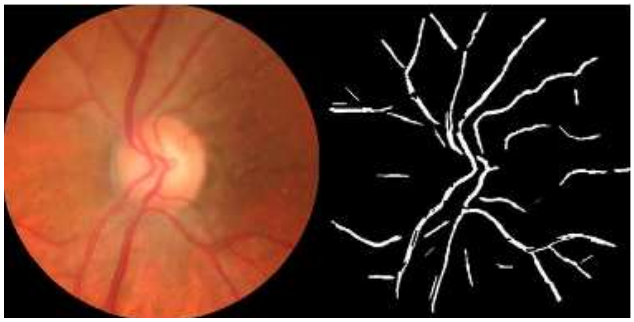
Liew *et al.* (Liew et al. 2008b) found the relationship between D_f and SBP to be even stronger than that of RVC and SBP, suggesting that fractal dimension may be a more sensitive marker of microvascular structural change than RVC. Variations in retinal vascular fractal dimension were also found to be inversely and significantly correlated with age (Liew et al. 2008b). More recently, deviations from optimal microvascular architecture (high or low D_f) have been shown to be associated with chronic kidney disease (CKD) (Sng et al. 2010). Independent of age, gender, ethnicity, diabetes, blood pressure, and other vascular risk factors, high (more complex) and low (less complex) retinal D_f was associated with increased risk of CKD. **Figure 12** shows examples of retinal vessel fractal patterns exhibiting **(A)** high vascular complexity, **(B)** intermediate vascular complexity, and **(C)** low vascular complexity.



(A) shows a D_f of 1.4958



(B) shows a D_f of 1.4541



(C) shows a D_f of 1.4073

Figure 12. **(A)** An eye with a high fractal dimension (D_f = 1.4958). **(B)** An eye with an intermediate fractal dimension (D_f = 1.4541). **(C)** An eye with a low fractal dimension (D_f = 1.4073). (Sng et al. 2010).

In a study examining the relationships between retinal vascular D_f and long-term diabetes-related micro and macro vascular complications in individuals with type 1 diabetes, retinal D_f was shown to be associated with microvascular complications of proliferative retinopathy and neuropathy, but not macrovascular disease (Grauslund et al. 2010). Despite positive findings, further studies examining systemic correlates, disease-predictive value, and responses to therapeutic interventions of retinal fractal analysis are clearly needed.

1.11 Exercise and Retinal Microvasculature

Individuals with type 2 diabetes experience impaired microvascular function (Middlebrooke et al. 2006). Jaap et al. (Jaap et al. 1995) found that individuals with type 2 diabetes have increased risk of microvascular complications, even in the absence of traditional microvascular complications of the disease. It has been suggested that microvascular dysfunction may be a key modulator in endothelium abnormalities seen in diabetic patients (Tooke and Hannemann 2000). While evidence suggests that regular exercise can improve macrovascular endothelial function in diabetic populations (for review see Sonne et al. 2007), evidence demonstrating improvements in microvascular endothelial function following regular exercise is limited (Middlebrooke et al. 2006). Hamburg et al. (Hamburg et al. 2007) reported that **physical inactivity** was associated with development of impaired microvascular function in healthy volunteers, suggesting that microvasculature is highly sensitive to metabolic changes associated with **physical inactivity**. To date, a few studies have examined the association between retinal vessel calibre and levels of physical activity/**inactivity** (Anuradha et al. 2011a; Anuradha et al. 2011b; Anuradha et al. 2011c; Tikellis et al. 2010). In the population-based Atherosclerosis Risk in Communities (ARIC) Study, lower levels of self-reported physical activity were shown to be significantly associated with wider venular calibre (Tikellis et al. 2010). In men, but not women, sedentary behaviour (prolonged television viewing time) has been shown to be associated with wider venular calibre (Anuradha et al. 2011a). No significant relationship was found between levels of physical activity and retinal vascular calibre in either gender. In the Multi-Ethnic Study of Atherosclerosis, lower levels of physical activity and higher levels of television viewing time were associated with wider retinal venular calibre (Anuradha et al. 2011b). In a multiethnic Asian population, lower physical activity and higher television time (in females) was associated with wider retinal venular calibre (Anuradha et al. 2011c). In these studies (Anuradha et al. 2011a; Anuradha et al. 2011b; Anuradha et al. 2011c; Tikellis et al. 2010), physical activity was assessed via self- report, which is often subject to recall bias and misclassification. In order to effectively assess the relationship between retinal

microvascular structure and physical activity, a more valid and reliable means of measuring varying levels of physical activity must be employed.

1.12 Modifiable Lifestyle and Environmental Risk Factors Affecting the Retinal Microcirculation

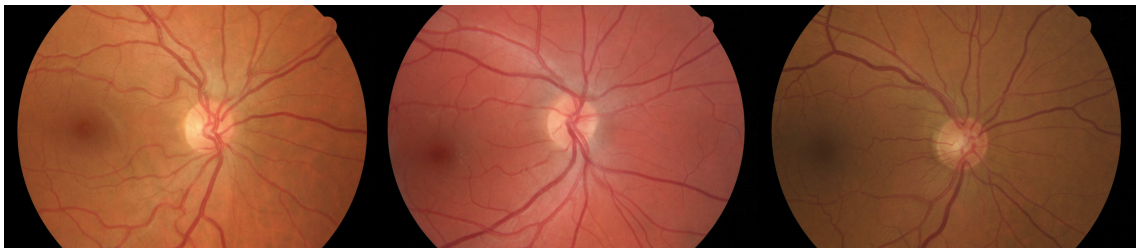
The retinal microcirculation may reflect healthy and pathophysiological processes affecting systemic circulation (Wong et al. 2005b). The vascular architecture within the retina, as well as elsewhere in the body, is thought to follow the principles of optimality, which allows the blood distribution to peripheral tissue within the quickest time with the least amount of energy (Murray 1926; Zamir 1976). Therefore, deviations from optimal structure of the retinal vasculature (e.g. arteriolar narrowing, venular widening) may represent deviation of the circulation from its optimal state, indicating pathophysiological processes.

During the last few decades, the retinal vasculature has received increasing attention. With the advancement of retinal imaging, the retinal vasculature may allow non-invasive visualization to examine and monitor human circulation systems in vivo (**Figure 13**). For example, computer-based analysis techniques from digital retinal images has allowed for accurate and reproducible measurement of several parameters of the retinal vasculature (e.g. calibre, fractal dimension [complexity of vessel network], and branching angle) (Benitez-Aguirre et al. 2011; Cheung et al. 2009; Liew et al. 2008c; Wong et al. 2001b; Wong et al. 2003). A number of large-scale epidemiological studies have demonstrated that subtle changes in these parameters carry important information regarding the future risk of systemic vascular diseases (Ikram et al. 2004; Klein et al. 2004; Klein et al. 2000; Liew et al. 2008c; Liew et al. 2011; Sun et al. 2008; Wong et al. 2001b; Wong et al. 2002a; Wong et al. 2006).

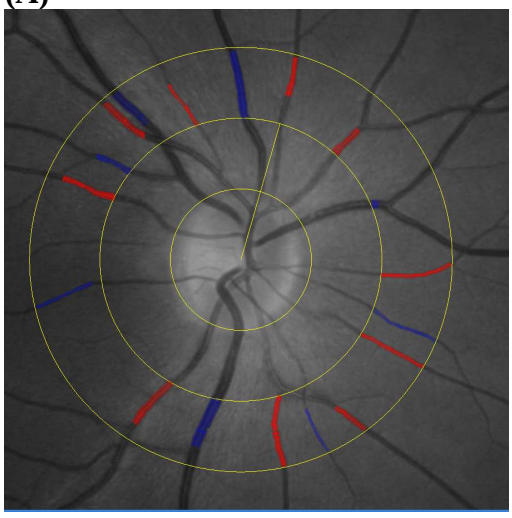
Importantly, changes in the retinal vasculature have also been shown to have strong associations with systemic and environmental cardiovascular risk factors in a range of populations (for review see Sun et al. 2009), even before the clinical manifestation of cardiovascular disease. These subtle retinal vascular changes have been suggested to mirror pre-clinical changes in both the cerebral (Kwa 2006) and coronary (Tedeschi-

Reiner et al. 2005) microcirculations. Although the mechanisms behind the association between retinal vascular changes and systemic cardiovascular risk factors have yet to be resolved, this relationship suggests that abnormalities in the retinal vasculature incorporate a cumulative effect of systemic damage.

Recently, many of the largest determinants of sub-optimal retinal microvasculature have been found to be modifiable (Liew et al. 2008c), such as diet and medications. More importantly, information regarding these modifiable exposures, together with measures of the retinal vasculature, may eventually provide clinically useful prognostic information regarding systemic disease risk prediction beyond current traditional risk factor assessment. In this review, we summarize recent research examining the modifiable lifestyle and environmental determinants affecting the retinal microvasculature and potential clinical implications of these findings.



(A)



(B)

Figure 13. **(A)** Examples of retinal vascular images with different morphologies, showing, from left to right, decreasing arteriolar calibre and vessel density. **(B)** Measurement of retinal arteriolar and venular calibre.

1.13 The Influence of Modifiable Lifestyle and Environmental Risk Factors on the Retinal Microvasculature

1.13.1 Diet

Dietary fibre intake, regular fish consumption, and low glycaemic index (GI) diets, such as those low in sugars and simple carbohydrates, are all associated with reduced risk of vascular disease (Brand-Miller et al. 2009; King 2005; Kris-Etherton et al. 2002). Emerging data suggest that the relationship between diet and macrovascular disease may partly be mediated by associated changes in the microcirculation (Kan et al. 2007; Kaushik et al. 2008; Kaushik et al. 2009). Work by Kan et al. (Kan et al. 2007) has shown that diet may have effects on RVC in the general population. For example, data from the ARIC study showed that higher intake of dietary fibre was independently associated with wider retinal arteriolar calibre and narrower venular calibre, indicating a lower risk of cardiovascular diseases (Kan et al. 2007). Similarly, findings from the Blue Mountain Eye Study (BMES) also demonstrated beneficial effects of increasing frequency of fish consumption on retinal microvasculature independent of other cardiovascular risk factors (Kaushik et al. 2008).

However, high-GI diets have been linked to deleterious anatomic changes in the retinal microvasculature (Kaushik et al. 2008; Kaushik et al. 2009). Kaushik et al. (Kaushik et al. 2009) found that high GI diets were associated with wider retinal venules and greater stroke mortality in persons 50 years and older. These findings suggest that post-prandial glucose may have deleterious effects on the cerebral microcirculation and may play a significant role in the relationship between diet and stroke mortality. More recent data from 823 schoolchildren aged 12.8 +/- 0.8 yr demonstrated that there was no association between a high-GI diet and retinal arteriolar or venular calibre (Lim et al. 2009). Evidence from the above study suggests a possible dose-dependent, cumulative effect of diet on the microvasculature over time.

The physiological influence of diet on the retinal microcirculation is complex. Kan et al. (Kan et al. 2007) found that the effect of fibre intake on retinal microvascular calibre might be confounded by concurrent hypertension and dislypidaemia. This suggests that

the beneficial retinal microvascular changes seen with increased fibre intake may not be directly effected by fibre intake itself, but by associated decreases in adverse systemic conditions like hypertension and dislypidaemia. For example, fish consumption is associated with increases in HDL (Bays et al. 2008). Increased concentration of HDL has a well-establish vasoprotective and anti-atherogenic effect (Miller 1987) and may alone explain the beneficial retinal microvascular changes associated with higher fish consumption. Findings demonstrating that the microvascular effects of diet were not evident in children free of systemic disease (Lim et al. 2009) supports this theory. Regular fish consumption, fibre intake, and low GI diets are all known to reduce systemic inflammation (Qi et al. 2006; Wall et al. 2010). Retinal microvascular changes are known to be affected by inflammatory factors (Klein et al. 2006b), and may be another biological mechanism through which diet mediates microvascular calibre. While the mechanisms underlying the above associations may not be completely understood, these data supports the vascular-protective effects of increased dietary fish, fibre, and low GI food consumption.

1.13.2 Physical Activity and Exercise

Sedentary behaviour, low levels of physical activity, and low cardiorespiratory fitness are all well-established risk factors for atherosclerosis and CVD (Laukkanen et al. 2002). Recent research has also shown the adverse effects of physical inactivity and low fitness extends to changes in microvascular structure (Anuradha et al. 2011a; Anuradha et al. 2011b; Anuradha et al. 2011c; Gopinath et al. 2011b; Hanssen et al. 2011; Tikellis et al. 2010). Sedentary behaviour, indicated by time spent watching television (TV), and lower levels of physical activity, assessed via self-report, were found to be associated with RVC (Anuradha et al. 2011a; Anuradha et al. 2011b; Anuradha et al. 2011c; Tikellis et al. 2010), suggesting a deleterious consequence of decreased levels of physical activity and increased sedentary behaviour upon the microvasculature. In addition, the impact of physical activity on the retinal microvasculature was also observed in a cohort of 6-year children. In the study by Gopinath et al., children who spent more time in outdoor sporting activities had wider mean RVC than those who spent more time watching TV (Gopinath et al. 2011b). More importantly, retinal arteriolar narrowing associated with

each hour of daily TV viewing time in children was similar in magnitude to that associated with a 10mm Hg increase in systolic blood pressure (Gopinath et al. 2011b).

Recently, evidence has been provided showing a relationship between higher levels of cardiovascular fitness and retinal microvascular structure (Hanssen et al. 2011). Higher cardiovascular fitness, as assessed by individual anaerobic threshold, was found to be associated with higher retinal arteriolar dilation and higher retinal AVR (Hanssen et al. 2011). In addition, ten weeks of exercise training was also shown to induce arteriolar dilatation in obese individuals and increased AVR in both obese and lean individuals (Hanssen et al. 2011). Conflicting results were found in a study of older women with type 2 diabetes in which no training-induced improvements in retinal vessel calibre were found after 12-weeks of moderate-intensity exercise (Serre, unpublished observation, 2011). In this cohort however, increased retinal microvascular density, shown by increased D_f was associated with increased time to exhaustion during incremental exercise testing (Serre, unpublished observation, 2011).

Observed associations between physical activity and changes in the retinal microvasculature may provide in vivo evidence regarding the effect of repeated bouts of regular exercise on the systemic circulation. While the exact pathophysiological mechanisms behind these relationships is not known, recent research suggests that moderators of vascular tone, specifically NO and ADMA, may play a significant role. ADMA, a NOS inhibitor, has been shown to directly affect retinal arteriole and venule diameters (Hemminki et al. 2007). More recently, Hanssen et al. (Hanssen et al. 2011) found that exercise training-induced increases in arteriolar calibre were accompanied by significant decreases in ADMA, suggesting the NO/ADMA pathway may play a key role in the beneficial changes in microvascular structure associated with regular exercise.

1.13.3 Obesity

The effect of obesity on the retinal microcirculation has been established (Cheung et al. 2007). Arteriolar calibre narrowing, venular calibre widening and lower AVR have been found to be associated with obesity in children and adult populations (Ikram et al. 2004; Klein et al. 2003; Klein et al. 2006b; Wang et al. 2006b; Wong et al. 2004a; Wong et al. 2006), suggesting that obesity may cause deleterious microvascular changes before clinical signs and symptoms of vascular disease are present. In children, a greater BMI was associated with wider retinal venular calibre and narrower arterioles, weight and body surface area were associated with wider retinal venules only, and larger waist circumference was associated with narrower retinal arterioles (Taylor et al. 2007).

In the Singapore Cohort Study of the Risk Factors for Myopia (SCORM) (Cheung et al. 2007), greater BMI and weight were associated with wider RVC. Consistent with this evidence, other recent studies have also demonstrated that BMI and triceps skinfold (Gopinath et al. 2011a; Li et al. 2011) were associated with wider RVC and narrower RVC in healthy, pre-adolescent children. Such evidence provides support for the theory that obesity may have an early adverse effect on microvascular structure.

While the mechanisms underlying the association between obesity and retinal vessel diameter are unclear, several possible explanations exist. Systemic inflammation is thought to contribute to the vascular complications associated with obesity (Berg and Scherer 2005). Systemic inflammation is also associated with changes in retinal venular calibre (Klein et al. 2006b), and therefore may be the mechanism which links obesity and changes in retinal microvascular structure. Obesity is also related to increased total blood volume (Oren et al. 1996), and retinal venular dilatation may be a regulatory response to maintain blood flow. These relationships between obesity and retinal microvascular changes may help explain the association between childhood obesity and complications such as hypertension, diabetes, and cardiovascular morbidity and mortality that occur later in life (Daniels 2009).

1.13.4 Cigarette Smoking

The Rotterdam study (Ikram et al. 2004), Beaver Dam Eye study (BDES) (Klein et al. 2006b), Multi-Ethnic Study of Atherosclerosis (MESA) (Wong et al. 2006), Wisconsin Epidemiologic Study of Diabetic Retinopathy (Klein et al. 2006b), and BMES (Kifley et al. 2007b) have all demonstrated a consistent association between wider retinal venular calibre and cigarette smoking, suggesting that adverse macrovascular outcomes associated with smoking may be partly mediated by deleterious changes in microvascular structure. More recently, the ARIC study has demonstrated a temporal association between past smoking and wider retinal venules, independent of current smoking status (Liew et al. 2008c), indicating that smoking may provoke long-term structural changes in microcirculation.

In a study examining the relative importance of current and past systemic determinants of RVC, it was found that current smoking surpassed mean arterial blood pressure, higher white blood cell count, BMI, and LDL cholesterol levels as the strongest determinant of wider RVC (Liew et al. 2008c). The use of RVC as a marker of damage from prolonged smoking has been strengthened by recent observations reporting retinal venular widening in patients with a history of smoking (Jeganathan 2005; Rosenberg et al. 2005).

Endothelial dysfunction and chronic inflammation have been shown to be associated with both RVC (Klein et al. 2006b; Wong et al. 2006) and smoking (Ambrose and Barua 2004; Landmesser et al. 2004; Michaud et al. 2006), and may partially explain the observed associations between RVC and smoking. Further longitudinal studies are required if the cumulative consequences of lifetime exposure to smoking, as well as a timeline for improvement in RVC changes after cessation of smoking, are to be determined. More importantly, additional research into the pathophysiology underlying the association between RVC and smoking is clearly needed.

1.13.5 Medications

The BDES (Wong et al. 2005b) and BMES (Leung et al. 2004; Liew et al. 2006) have examined the impact of specific medication use on retinal microvascular structure. These studies found associations between topical beta-blockers and retinal arteriolar and venular narrowing (Leung et al. 2004), and hormone replacement therapy and lower AVR (Liew et al. 2006). In the study by Thom et al. (Thom et al. 2009), it was shown that hypertensive patients receiving the calcium channel blocker, amlodipine besylate (Norvasc) had narrower arterioles than those receiving the beta-blocker atenolol. While these data suggest that anti-hypertensive treatment may prove useful in decreasing retinal arteriole narrowing due to hypertension, the effects of lowered BP were not accounted for. Generalized arteriole narrowing has been shown to be associated with past elevated blood pressure levels (Leung et al. 2003) and any relationship between anti-hypertensive medication and retinal microvascular structure may be due to associated decreases in blood pressure.

In order to examine the effects of angiotensin-convering enzyme inhibitor (ACEI) and angiotensin-receptor blocker (ARB) therapy on retinal vessel diameter, Klein et al. (Klein et al. 2010) examined a cohort of normo-tensive individuals with type 1 diabetes receiving anti-hypertensive treatment. No significant effect of ACEIs or ARBs on retinal vessel calibre was found in this population. The lack of effect of anti-hypertensive treatment on retinal vessel calibre suggests that the beneficial effects of anti-hypertensive treatment on the retinal microcirculation may be limited to those individuals whose retinal arterioles would likely be narrowed at baseline, and any relationship may be mediated by associated reductions in blood pressure. Further studies, including healthy control subjects, are required to determine if anti-hypertensive medications have an effect on retinal microcirculation when controlling for improvements in systemic diseases. If certain medications are found to have direct beneficial effects on retinal microvascular structure, targeted therapeutic interventions may be used to manage pre-clinical signs of systemic disease.

1.13.6 Environment

Epidemiological studies have demonstrated significant increases in cardiovascular morbidity and mortality with increased long- and short-tem exposure to air pollution (Brook et al. 2010). Impaired microvascular function has been suggested to play a role in the above associations. Recently, data from the population-based MESA has shown that retinal arteriolar calibre was narrower and RVC was wider among persons living in areas with increased long- and short-term exposure to fine particulate matter ($PM_{2.5}$) (Adar et al. 2010). A 3 ug/m^3 increase in $PM_{2.5}$ concentration was associated with arteriole narrowing equivalent to those seen with an age increase of seven years, a more traditional cardiovascular risk factor. The above association suggest that important vascular changes occur with small increases in long- and short-term exposure to air pollution.

A wider venular diameter with chronic air pollution exposure is consistent with similar findings from studies examining the effects of smoking on retinal microvascular structure (Ikram et al. 2004; Jeganathan 2005; Kifley et al. 2007a; Klein et al. 2006a; Klein et al. 2006b; Liew et al. 2008a; Rosenberg et al. 2005; Wong et al. 2006), and may be mediated in part by inflammation-related mechanisms. Long-term exposure to air pollution is known to promote inflammation and endothelial dysfunction (Briet et al. 2007; Simkhovich et al. 2008), and may lead to disruption of the microvascular autoregulatory function and venular widening within the retina. Practically, the above findings are important in that sub-clinical microvascular changes (arteriolar narrowing and venular widening) were reported in individuals exposed to $PM_{2.5}$ levels, well below established regulatory thresholds (Adar et al. 2010). These data may provide information necessary to establish safer and more accurate regulatory air quality standards.

1.14 Conclusions

Recent work on selected modifiable risk factors and the retinal microcirculation has provided evidence relating modifiable lifestyle and environmental risk factors to adverse cardiovascular outcomes. Exposure to modifiable risk factors may affect systemic physiology, which is reflected in changes in the retinal microvascular structure. The

retina is an easily accessible site in which the human microcirculation can be visualized non-invasively and quantified. The potential use of retinal imaging as a biomarker of reversible pathophysiological processes within the systemic circulation is promising.

Nevertheless, evidence showing that quantitative assessment of retinal microvasculature can provide prognostic information beyond the current traditional risk factors is limited. Currently, there have been no established reference levels for age, gender, or disease status, which therefore still limits the utility of retinal imaging as a tool to monitor cardiovascular and metabolic risk in asymptomatic patients or those who have other traditional, positive risk factors.

More longitudinal studies are needed to determine if changes in retinal microvascular structure can be positively modified with various therapeutic interventions (e.g. diet, exercise). Retinal imaging and interpretation may provide clinicians with a diagnostic tool to assess the effects of specific interventions on disease progression. Research into the possible prognostic value of retinal imaging, and subsequent interpretation, in subjects without clinically evident systemic diseases, e.g. healthy children, may provide evidence of clinically significant associations between retinal vascular structure and disease risk factors. Long-term systemic disease risk stratification in childhood may provide clinicians with information necessary to target microvascular risk factors in therapeutic interventions, and before overt signs of systemic diseases become evident. Advancing our understanding of the pathophysiology behind changes in retinal microvascular structure in diseased states may aid in the development of novel prediction and intervention strategies for a range of systemic conditions. Although retinal imaging shows promise as a powerful clinical tool, further epidemiologic research is needed if retinal imaging is to become widely used in disease-risk identification and management.

Table 2. Summary of studies examining the modifiable lifestyle and environmental determinants affecting the retinal microvasculature. (Serre and Sasongko 2011).

Risk Factors	Authors	Year of Publication	Population	Measured Exposure	Observed Outcome
Diet	Kan et al.	2007	10,659 US adults (ARIC)	Increased dietary fibre	Wider retinal arteriolar and narrower venular calibre
Diet	Kaushik et al.	2008	3,654 adults aged 49+ years (BMES)	Increased Fish consumption	Wider retinal arteriolar and narrower venular calibre
Diet	Kaushik et al.	2009	3,654 adults aged 49+ years (BMES)	High Glycemic Index	Wider retinal venular calibre
Diet	Lim et al.	2009	823 Singapore School Children 12.8 +/- 0.8 yrs	High Glycemic Index	No significant association
Physical Activity	Gopinath et al.	2011b	2238 six-year-old Sydney students	Increased sporting activities	Wider retinal arteriolar calibre
				Increased TV viewing time	Narrower retinal arteriolar calibre
Physical Activity	Anuradha et al.	2011a	2,024 Australian adults (AusDiab)	Low self-reported physical activity	No significant association
				Increased self-reported TV viewing time	Wider retinal venular calibre
Physical Activity	Anuradha et al.	2011b	5,893 US adults from 4 racial/ethnic groups (MESA)	Low self-reported physical activity	Wider retinal venular calibre
				Increased self-reported TV viewing time	Wider retinal venular calibre
Physical Activity	Tikellis et al.	2010	15,792 US adults 45-64 years (ARIC)	Higher self-reported physical activity	Wider retinal venular calibre
Cardiovascular Fitness	Hassen et al.	2011	46 adult recreational runners	Higher cardiovascular fitness (AT)	Wider retinal arteriolar calibre and higher AVR
				10-week exercise program	Retinal arteriolar dilatation in obese individuals
Cardiovascular Fitness	Serre et al.	Unpublished observations	15 women 65-74 years with type 2 diabetes	12-week exercise program	No significant changes
Cardiovascular Fitness	Serre et al.	Unpublished observations	15 women 65-74 years with type 2 diabetes	Increased time to exhaustion during graded exercise testing	Increased fractal dimension
Obesity	Taylor et al.	2007	1,740 Sydney school children (SCES)	Increased BMI & weight	Wider retinal venular calibre
				Increase BMI & waist circumference	Narrower retinal arteriolar calibre
Obesity	Cheung et al.	2007	768 Singapore school children (SCORM)	Increased BMI & weight	Wider retinal venular calibre
Obesity	Gopinath et al.	2011a	3,144 Sydney school children	Increased BMI	Narrower retinal arteriolar and wider venular calibre
Obesity	Li et al.	2011	136 Singapore school children (STARS)	Increased triceps skinfold and BMI	Wider retinal venular calibre
Cigarette Smoking	Liew et al.	2008	15,792 US adults 45-64 years (ARIC)	Past cigarette smoking	Wider retinal venular calibre
Cigarette Smoking	Rosenburg	2005	Clinical observation	Past and current cigarette smoking	Clinical retinal venular widening
Cigarette Smoking	Jeganathan	2005	Clinical observation	Past and current cigarette smoking	Clinical retinal venular widening
Medication	Thom et al.	2009	19,342 adults 40-79 years undergoing antihypertensive therapy (ASCOT)	Calcium channel blocker vs. β-blocker therapy	Less retinal arteriole narrowing with calcium channel blocker therapy
Medication	Klein et al.	2010	147 adults with type 1 diabetes	ACEI and ARB therapy	No significant changes
Air Pollution	Adar et al.	2010	6,814 adults 45-84 years (MESA)	Higher long- & short-term $PM_{2.5}$ exposure	Narrower retinal arteriolar calibre

1.15 The Overall Aim of the Proposed Thesis:

Overall aim of the proposed thesis is:

(1) To examine select responses in vascular structure (RVC and D_f) to twelve weeks of controlled and supervised exercise training in women aged 65-74 yr with and without uncomplicated type 2 diabetes (no associated peripheral neuropathy, nephropathy and/or Peripheral Arterial Disease) when the total exercise time (120 minutes per week) and relative exercise intensity (T_{ge}: gas exchange threshold) are held constant.

Specific aims of the proposed studies are to:

(1) (Study 1) - Examine the relationship between retinal vessel calibre/fractal measurements and measures of physiological functional capacity and endothelial function in women aged 65-74 yr with and without uncomplicated type 2 diabetes.

(2) (Study 2) - Examine changes in retinal vessel calibre and fractal analysis in women aged 65-74 yr with and without uncomplicated type 2 diabetes, following twelve weeks of controlled and supervised exercise training at T_{ge} (60-65% VO_2 peak), for a total exercise time of 120 minutes per week.

(3) (Study 3) - Examine the effects of twelve weeks of controlled and supervised exercise training at T_{ge} (60-65% VO_2 peak), for a total exercise time of 120 minutes per week, on plasma ADMA concentration in women aged 65-74 yr with uncomplicated type 2 diabetes mellitus.

CHAPTER 2 – *Study One*

Physiological Functional Capacity and Retinal Microvascular Morphology in Women Aged 65-74 Yr with and without Type 2 Diabetes

2.1 Introduction

Physiological functional capacity (PFC) has been previously defined as the ability to perform the physical tasks of daily life and the ease with which these tasks can be performed (Tanaka and Seals 2003). PFC is intended to reflect the central and peripheral components that mediate oxygen supply and utilization. Decreased PFC is a well-established risk factor for cardiovascular disease and all-cause mortality (Tanaka and Seals 2003). The health benefits that accompany an increase in peak oxygen uptake (VO_2peak) are well described (Pate et al. 1995) and include significant beneficial changes to endothelial function and to the macrocirculation (Haddock et al. 1998; Kemi et al. 2004; Lakka et al. 2001). The relationship between measures of PFC and microcirculatory function is not as well understood, partly due to the difficulty of examining the microcirculation *in vivo*.

The retinal microvasculature can provide important clinical information on the state of the microcirculation in the retina and on the cumulative cerebral and coronary vascular damage resulting from aging, hypertension, and other disease processes (Liew et al. 2007). Given that regular physical activity is well established as an effective means of decreasing vascular damage associated with the above conditions, it is reasonable to assume that measures of PFC may be associated with the retinal microcirculation. Few studies have examined the relationship between measures of PFC and retinal microvascular structure. Wider venular calibre has been associated with lower levels of physical activity (Anuradha et al. 2011b; Anuradha et al. 2011c; Tikellis et al. 2010) and sedentary behaviour in men, but not in women (Anuradha et al. 2011a). In these studies, levels of physical activity and inactivity were estimated via self-reported questionnaires. To date, the relationship between more direct measures of PFC [including VO_2peak, peak heart rate, respiratory exchange ratio (RER) and time to exhaustion (TE)] and structure of the retinal microcirculation has not been examined. Thus, it is unknown whether moderate-intensity exercise has any longitudinal beneficial effects on the retinal microcirculation.

Also, previous studies examining a possible relationship between PFC and microvascular structure and function have used RVC as a measure of systemic microvascular structure.

Fractal analysis (D_f) has recently been used to summarize the geometric complexity of retinal vasculature, providing a more “global” and sensitive measure of microvascular structure.

The aim of the present study was to determine the relationship between measures of PFC and variations in RVC and retinal D_f in females aged 65-74 yr with and without uncomplicated type 2 diabetes. Appropriately prescribed exercise could improve the structure and function of the microvasculature in those persons with and without uncomplicated type 2 diabetes.

2.2 Methods

2.2.1 Subjects and Recruitment Strategies

Forty female subjects (19 Type 2 diabetic and 21 non-diabetic), aged 65-74 years participated. Subjects were recruited from local GP clinics, media advertisements, and mail-outs by Diabetes Australia Queensland. Potential subjects were initially screened by telephone interview using a standardized set of questions to determine suitability to enter a formal health screening. The following inclusion criteria were used: (i) clinical diagnosis of type 2 diabetes for at least 12 months (for type 2 diabetic subjects), (ii) not currently using exogenous insulin (iii) non-smoking, (iv) normal age-relative physical examination, (v) no severe musculoskeletal disability, and (vi) not currently taking medications known to directly interfere with exercise responses (i.e. beta blockers or warfarin). All subjects provided written consent to participate. Each subject's cognitive capacity to give consent was determined using the Mini-Mental State Examination (Folstein et al. 1975) and assessed by an independent observer. During the health-screening process, height was measured (in millimetres) using a wall-mounted stadiometer (Harpenden, Holtain Ltd, Crosswell, UK). Body mass was measured using a calibrated digital scale (CH-150k, A & D Pty Ltd, Thebarton, Australia) and waist and hip circumference were measured. Pulmonary function was also assessed by measurements of forced vital capacity and forced expiratory volume in 1 second using a calibrated spirometry system (Ultima CPX, Medical Graphics Corporation, St Paul,

USA). Supine and standing 12-lead ECG (Cardio Perfect, Welch Allyn Inc., Skaneateles Falls, USA) and blood pressure were measured at rest.

After an overnight fast, venous blood samples were drawn (between 0700 – 0830 hours) at an accredited pathology laboratory for glucose, insulin, HbA_{1c} (for type 2 diabetic subjects), low- and high-density lipoprotein cholesterol, triglycerides and homocysteine measurements (**Appendix E**). Subjects were then asked to visit their family physician with the health-screening results and details of the experimental procedures for an opinion about the suitability of our patient to participate. Bond University Human Research Ethics Committee and Griffith University Human Ethics Committee reviewed and approved the experimental protocol (RO-745).

2.2.2 Determination of Peak Oxygen Uptake and Gas-Exchange Threshold

Subjects completed an incremental exercise test to volitional fatigue on a motor driven treadmill ('Valiant'; Lode B.V., Groningen, Netherlands) to determine peak oxygen uptake (VO_2peak), time to exhaustion (TE) and the gas exchange threshold (T_{ge}). Before the exercise testing session, subjects were familiarized with treadmill walking at various speeds (2.0 – 6.0 km h^{-1}) and treadmill grades (0 – 6%). Each subject's preferred walking speed was determined (at 1% grade). The subject's preferred walking speed was used during all subsequent exercise tests and training sessions. The incremental exercise test consisted of a 4-min warm-up at 3.0 km·h^{-1} and 1% grade, followed by a speed increase every minute until the previously determined self-selected walking speed was attained. Treadmill grade was then increased by 2% every minute until volitional fatigue or clinically significant signs or symptoms precluded further exercise. Cardiac rate and rhythm was monitored continuously using a 12-lead ECG and brachial artery blood pressure was measured by auscultation every 3 minutes during the incremental exercise test. Carbon dioxide output (VCO_2), oxygen uptake (VO_2), and expired minute ventilation (V_E BTPS) were measured breath-by-breath and averaged every 30 seconds using an open-circuit metabolic measurement system (Ultima CPX, Medical Graphics Corporation, St Paul, USA). The gas analysers and pneumotachograph were calibrated before and after each incremental exercise test using precision reference gases and an independently calibrated 3-L calibration syringe (Hans Rudolph Inc., Kansas City, USA).

Peak gas exchange variables (VO_2, VCO_2) are reported as the average of the two highest consecutive 30-second values measured before volitional fatigue. The T_{ge} was determined using the simplified V-slope method (Schneider et al. 1993).

2.2.3 Retinal Photography and Measurement of Retinal Vascular Calibre

After pupil dilation, colour static digital fundus photographs (2 fields of each eye, one centred on the optic disk and a second on the macula) were taken using a 45° 6.1 megapixel digital non-mydriatic camera (Nikon D100, Nikon Corporation, Japan). Retinal photographs were taken within 24-48 hours of the incremental exercise test. Images were graded by the Retinal Vascular Imaging Centre (RetVIC), Centre for Eye Research, Melbourne, Australia for measurements of retinal vascular morphology.

Retinal vascular calibre was measured by trained investigators blinded to participant characteristics using a computer-based program (Retinal Analysis, University of Wisconsin, Madison, WI, USA) (Liew et al. 2007; Wong et al. 2004b). Briefly, the diameter of all arterioles and venules within a concentric zone corresponding to a half- to one-disc diameter away from the optic disc margin were measured. Diameters of the widest six arterioles and venules were summarized as the central retinal artery equivalent (CRAE) and the central retinal venular equivalent (CRVE). CRAE and CRVE represent the estimated average calibre of the central retinal artery and vein, respectively (Knudtson et al. 2003). Only retinal images from the right eye were analysed. Intra- and inter-grader reproducibility of retinal vascular measurements performed at this site have previously been reported, with intra-class correlation coefficients ranging from 0.78 to 0.99 (Wong et al. 2004b). The intra-class correlation coefficient for the present study, based on re-grading of 20 randomly chosen photographs ranged from 0.79 to 0.98.

2.2.4 Measurement of Retinal Fractal Dimension

Fractal analysis was completed by trained investigators blinded to participant characteristics, using a computer-based program (International Retinal Imaging Software [IRIS], National University of Singapore, University of Melbourne, University of Sydney; Patent pending), which measured D_f (Liew et al. 2008b). Retinal vascular D_f of each image was calculated within a 3.5-disc radii pre-defined circular area centred on the

optic disk. Retinal vessels within this region were automatically traced by the software and subsequently examined by investigators to identify and erase artefacts (e.g., peri-papillary atrophy, choroidal vessels, and pigment abnormalities) that could be mistaken by the software as retinal vessels. Fractal analysis was then performed automatically by the IRIS software and D_f calculated using the box counting approach (Stosic and Stosic 2006). Reliability of the IRIS measurements was high, with intra-grader intra-class correlation coefficients ranging from 0.93 to 0.94.

2.2.5 Statistical Analysis

Results are reported as mean ± standard error unless otherwise stated. An independent t-test was used to determine difference in means during between group comparisons. Bivariate correlations were performed to detect significant relationships between dependent variables after adjusting for mean arterial blood pressure (MAP). All statistical differences were accepted at the $p < 0.05$ level. SPSS (SPSS Inc, Release 17.0, USA) was used.

2.3 Results

Forty subjects completed the study. One participant with type 2 diabetes was excluded as retinal vessel calibre could not be measured in either eye. The descriptive characteristics and blood profiles of the subjects are shown in **Table 1** and **2**.

2.3.1 Age, Blood pressure, and Anthropometric Variables

Subjects with type 2 diabetes had significantly higher body mass, BMI, waist-hip ratio, and resting systolic blood pressure when compared to age- and sex-matched non-diabetic subjects (**Table 1**).

2.3.2 Lipid Profile, Blood Glucose, and Haematology

The concentration of fasting glucose and insulin were significantly higher, and HDL was significantly lower, in those subjects with type 2 diabetes (**Table 2**). Total cholesterol and LDL were significantly higher in non-diabetic subjects (**Table 2**). Total white cell count, neutrophil and lymphocyte concentrations were significantly higher in those subjects with

type 2 diabetes. There were no significant differences found in any other haematological values (**Table 3)**.

2.3.3 Physiological Functional Capacity

Subjects with type 2 diabetes had significantly lower VO_2peak, peak heart rate, RER and TE. There were no significant differences between the two groups in T_{ge} (expressed as a percentage of VO_2peak) (**Table 4)**.

2.3.4 Retinal Vessel Calibre and Fractal Analysis

There were no significant differences between groups in CRAE (with diabetes: 153.3±3.8 μm vs. without diabetes: 154.7±2.7 μm, p = 0.760), CRVE (with diabetes: 220.2±4.4 μm vs. without diabetes: 230.8±4.9 μm, p = 0.121), or D_f (with diabetes: 1.45 ± 0.004 vs. without diabetes: 1.45±0.004, p = 0.595).

2.3.5 Relationships between Retinal Vessel Calibre, Fractal Analysis, and Gas Exchange Data

There were no significant relationships between CRAE or CRVE and any measure of PFC (VO_2peak, TE, peak RER, peak HR, $V_E$peak, or T_{ge}) in either group. D_f was found to be significantly correlated with TE only in those with type 2 diabetes ($r = 0.48$, $p = 0.04$) (**Figure 1**). No significant correlations were found between D_f and any other measures of PFC.

2.4 Discussion

The principal findings of the present study were: (i) no significant differences were found between type 2 diabetic and non-diabetic groups in CRAE, CRVE, or D_f; and (ii) D_f was found to be significantly correlated with TE in the type 2 diabetic group.

Non-diabetic subjects in the present study had similar anthropometric characteristics and blood profiles as those reported elsewhere (Carvalho et al. 2010; De Vito et al. 1999). Individuals with type 2 diabetes displayed significantly higher body mass, BMI, waist-

hip ratio, resting systolic blood pressure, plasma glucose and insulin concentration commonly associated with this disease (American Diabetes Association 2012a). Individuals with type 2 diabetes also had significantly increased white cell count and neutrophil and lymphocyte concentrations compared to non-diabetic individuals, which has been reported elsewhere (Gkrania-Klotsas et al. 2010). While individuals with type 2 diabetes in the present study had similar anthropometric characteristics to age- and sex-matched subjects in other studies, cholesterol and LDL levels were significantly lower (McGavock et al. 2004; Onisto et al. 2009). Subjects with type 2 diabetes participating in the present study were medically well managed, with the majority of subjects also being treated for elevated cholesterol. None of the 20 non-diabetic subjects were taking lipid-lowering medication. The aggressive lipid lowering treatment received by the majority of subjects with type 2 diabetes in the present study may explain the lower levels of cholesterol and LDL than age- and sex-matched non-diabetic subjects.

Subjects with type 2 diabetes had significantly lower measures of physiological functional capacity compared to the non-diabetic group, a well-established consequence of type 2 diabetes (Regensteiner et al. 1995). While the exact cause of the decrease in PFC that accompanies type 2 diabetes is unknown, it has been suggested that genetically determined low mitochondrial number may decrease an individual's VO_2peak (Leite et al. 2009). Subjects with type 2 diabetes in our study had similar VO_2peak values as age- and sex-matched individuals with type 2 diabetes in previous studies (McGavock et al. 2004; Walker et al. 1999).

No significant differences in retinal vessel calibre or fractal dimension were found in subjects with and without type 2 diabetes, which is in contrast to the results of others (Wong et al. 2002b; Wong et al. 2005b; Yau et al. 2010). Previous studies found individuals with type 2 diabetes had narrower retinal arteriolar diameter (Wong et al. 2002b; Wong et al. 2005b) and greater retinal D_f (Yau et al. 2010) than non-diabetic individuals. A possible reason for this difference may be that individuals with type 2 diabetes participating in our study were medically well-managed and the concomitant pharmaceutical interventions may have protected these individuals against significant microvascular structural changes. Antihypertensive medication, taken by the majority of

the individuals with type 2 diabetes in our study, has previously been shown to have beneficial effects on retinal microcirculation (Thom et al. 2009).

No significant relationship was found between any measures of PFC and retinal vessel calibre in either cohort. While no previous studies have investigated the relationship between direct measures of PFC and retinal microvascular structure, there is emerging evidence that significant relationships are observed between retinal vessel calibre and levels of physical activity and sedentary behaviour (Anuradha et al. 2011a; Anuradha et al. 2011b; Anuradha et al. 2011c; Tikellis et al. 2010). In the population-based ARIC Study, lower levels of self-reported physical activity were significantly associated with wider venular calibre (Tikellis et al. 2010). In men, but not women, sedentary behaviour, as determined from prolonged television viewing time, was associated with wider venular calibre (Anuradha et al. 2011a). There was no significant relationship between self-reported levels of physical activity and retinal vascular calibre (Anuradha et al. 2011a). Lower levels of physical activity were associated with wider retinal venular calibre in the Multi-Ethnic Study of Atherosclerosis (Anuradha et al. 2011b). In these studies (Anuradha et al. 2011a; Anuradha et al. 2011b; Anuradha et al. 2011c; Tikellis et al. 2010) physical activity was assessed via a self-report; such methods of determining a physical activity history are often inaccurate (Sallis and Saelens 2000). The present study assessed PFC by controlled, clinical exercise testing using established methods and measurement techniques with known validity and reliability. Studies that have examined the relationship between cutaneous microvascular function and direct measures of PFC have been inconclusive (Boegli et al. 2003; Lenasi and Strucl 2004; Pasqualini et al. 2010; Tew et al. 2010; Vassalle et al. 2003). It now appears that only studies examining cohorts with large differences in VO_2max, showed significant relationships between measures of PFC and cutaneous microvascular function (Lenasi and Strucl 2004; Pasqualini et al. 2010; Tew et al. 2010; Vassalle et al. 2003). Studies consisting of a relatively homogenous cohort with regards to VO_2max showed no such relationships (Boegli et al. 2003). In our study, participant pool was relatively homogenous with regards to measures of PFC in individuals with and without type 2 diabetes, which may have masked possible correlations with retinal vessel calibre. Further research may

require a larger, more diverse population with regards to measures of PFC to accurately assess this relationship.

Prior to our study, the relationships between measures of PFC and D_f were unknown. Unlike quantifying microvascular structure using retinal vessel diameter, fractal analysis allows for a more global measure of retinal vascular pattern and its efficiency in circulation (Kamiya and Takahashi 2007; Takahashi et al. 2009). Quantifying retinal vascular complexity using retinal fractal dimensions may provide a more accurate and sensitive overall estimate of retinal microvascular structure (Liew et al. 2008b). In our study, subjects with type 2 diabetes, higher D_f was found to be positively correlated with time to exhaustion during maximal exercise testing. D_f likely provided a more accurate measure of retinal microvascular structure than retinal vessel calibre, displaying correlations with measures of PFC that may have been masked by a less sensitive measure like retinal vessel calibre.

Our study is the first to examine, and show, associations between measures of retinal vasculature structure and measures of PFC. At this time we are unable to provide a mechanism for the significant relationship between PFC and D_f. The beneficial vascular adaptations to regular exercise have been shown to be largely mediated by changes in vascular structure (Prior et al. 2004), specifically in improvements in endothelial function (Vassalle et al. 2003). It has been suggested that changes in retinal microvascular structure result from underlying endothelial dysfunction, and may prove to be a effective surrogate maker of endothelial function (Liew et al. 2008c). Therefore, exercise-induced changes in endothelial function may contribute to the associations between D_f and measures of PFC in our study. Further research is needed to understand the possible differential relationship between D_f and measures of PFC in diabetic and non-diabetic populations.

In conclusion, we report novel associations between retinal vascular fractal dimension and time to exhaustion in women 65-74 years with type 2 diabetes. Further investigation involving a larger, more diverse population with regards to VO_2peak/max is required to determine the relationship between retinal microvascular structure and measures of PFC.

Table 1. Characteristics of participating subjects with and without type 2 diabetes. Mean ± SEM.

	With Type 2 Diabetes (*N*=19)	Without Type 2 Diabetes (*N*=20)
Subject characteristics		
Age (yr)	68.9±0.78	67.3±0.73
Body Mass (kg)∗	76.2±3.4	68.3±1.9
BMI ($kg \cdot m^{-2}$)∗	29.9±1.1	25.9±0.7
Waist/Hip Ratio∗	0.85±0.02	0.78±0.02
SBP (mmHg)∗	133±3	124±3
DBP (mmHg)	74±2	73±1

∗ Significant difference found between individuals with and without type 2 diabetes ($p < 0.05$)
BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure.

Table 2. Blood profiles of participating subjects with and without type 2 diabetes. Mean ± SEM.

	With Type 2 Diabetes (*n*=19)	Without Type 2 Diabetes (*n*=20)
Blood Profile		
Homocysteine ($\mu mol \cdot L^{-1}$)	12.1±1.3	10.3±0.7
Glucose ($mmol \cdot L^{-1}$)∗	7.4±0.4	5.1±0.1
HbA_{1C} (%)	6.6±0.2	N/A
Insulin ($mU \cdot L^{-1}$)∗	10.9±2.2	5.1±0.6
Cholesterol ($mmol \cdot L^{-1}$)∗	4.2±0.2	5.7±0.2
Triglycerides ($mmol \cdot L^{-1}$)	1.3±0.1	1.1±0.1
HDL ($mmol \cdot L^{-1}$)∗	1.3±0.1	1.8±0.1
LDL ($mmol \cdot L^{-1}$)∗	2.3±0.2	3.5±0.2

∗ Significant difference found between individuals with and without type 2 diabetes ($p < 0.05$)
HbA_{1c}, haemoglobin A_{1c}; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

Table 3. Haematological characteristics of participating subjects with and without type 2 diabetes. Mean ± SEM.

	With Type 2 Diabetes (*n*=19)	Without Type 2 Diabetes (*n*=20)
Haemoglobin ($g \cdot L^{-1}$)	129.4±4.1	131.05±1.9
Haematocrit (%)	40±1	40±1
Red cell count ($10^{12} \cdot L^{-1}$)	4.39±0.1	4.37±0.1
White cell count ($10^{9} \cdot L^{-1}$) ∗	6.63±0.3	5.25±0.3
Neutrophils ($10^{9} \cdot L^{-1}$) ∗	3.46±0.3	2.69±0.2
Lymphocytes ($10^{9} \cdot L^{-1}$) ∗	2.37±0.2	1.88±0.1
Monocytes ($10^{9} \cdot L^{-1}$)	0.57±0.04	0.48±0.03
Eosinophils ($10^{9} \cdot L^{-1}$)	0.24±0.1	0.17±0.02
Basophils ($10^{9} \cdot L^{-1}$)	0.03±0.004	0.03±0.004
Albumin ($g \cdot L^{-1}$)	39.5±2.9	42.8±0.6

∗ Significant difference found between subjects with and without type 2 diabetes ($p < 0.05$)

Table 4. Gas exchange data for participating subjects with and without type 2 diabetes. Mean ± SEM.

	With Type 2 Diabetes (*n*=19)	Without Type 2 Diabetes (*n*=20)
TE (min) ∗	13.2±0.7	17.1±0.5
VO_2 peak ($L \cdot min^{-1}$) ∗	1.48±0.04	1.64±0.1
VO_2 peak ($mL \cdot kg^{-1} \cdot min^{-1}$) ∗	20.0±0.9	24.3±1
Peak HR ($b \cdot min^{-1}$) ∗	144±4	159±3
V_E peak($L \cdot min^{-1}$)	51.9±2.4	56.8±1
T_{ge} (%VO_2 peak)	70±1	69±1
Peak RER ∗	1.1±0.02	1.2±0.02

∗ Significant difference found between participating subjects with and without type 2 diabetes ($p < 0.05$)
TE, time to exhaustion; VO_2 peak, peak oxygen uptake; HR, heart rate; V_E, ventilation; T_{ge} gas exchange threshold; Peak RER, peak respiratory exchange threshold.

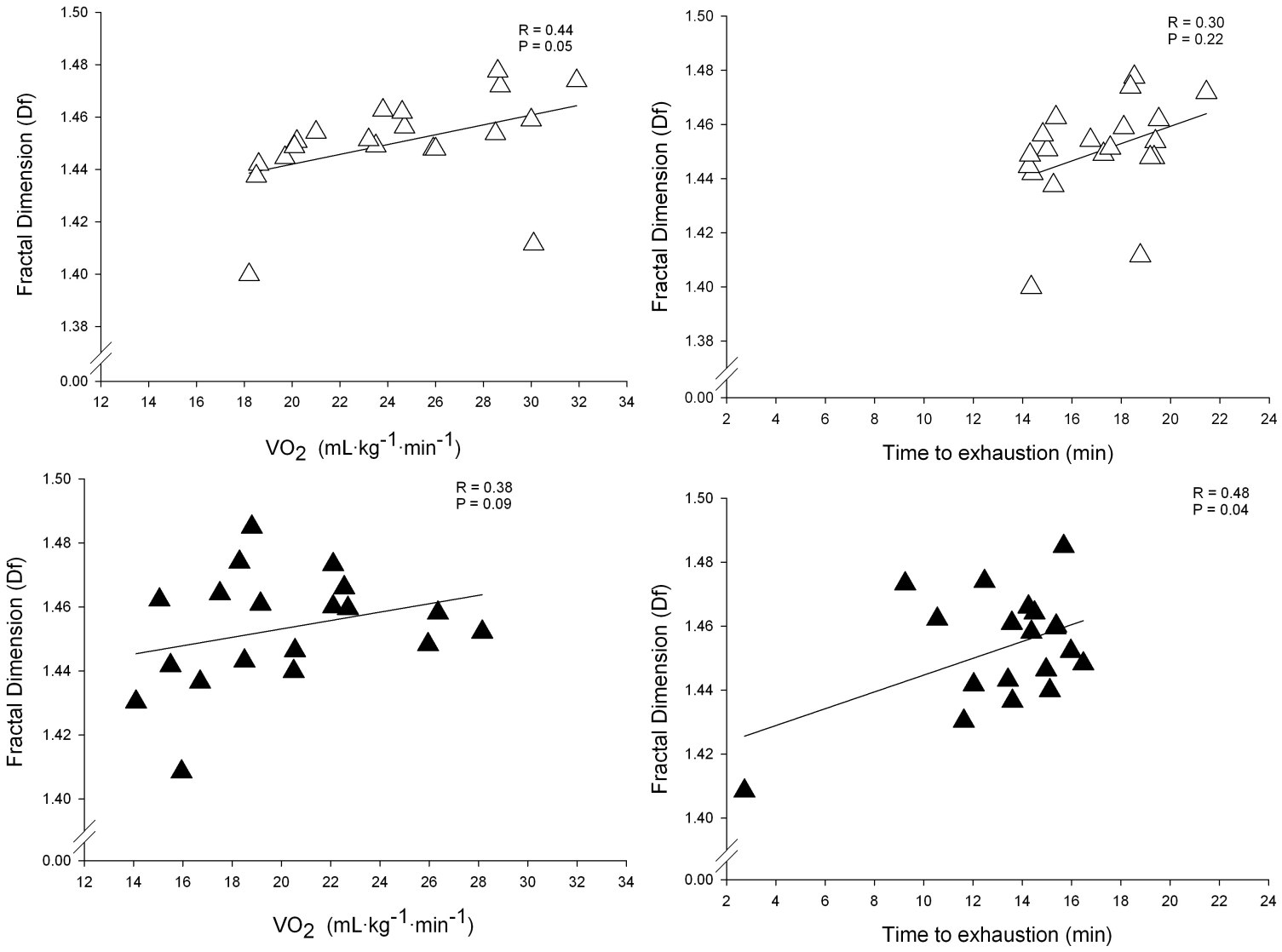


Figure 1. Fractal dimension, VO_2, and time to exhaustion in participating subjects without (Δ) and with (▲) type 2 diabetes. R is the Pearson product–moment correlation coefficient.

CHAPTER 3 - *Study Two*

Retinal Microvascular Responses to Exercise in Women with Type 2 Diabetes

3.1 Introduction

Increased risk of cardiovascular disease and microvascular dysfunction are well-established co-morbidities of type 2 diabetes. While numerous pharmacologic agents have been shown to effectively moderate cardiovascular disease risk associated with type 2 diabetes, lifestyle modification, including regular exercise, remains an effective therapeutic intervention for individuals with type 2 diabetes (Marwick et al. 2009). Regular exercise is effective in improving macrovascular endothelial function in persons with type 2 diabetes (Maiorana et al. 2001) and in older individuals (DeSouza et al. 2000). A few studies have investigated and reported the effects of repeated bouts of exercise on cutaneous microvascular structure (Black et al. 2008; Goto et al. 2003; Hamdy et al. 2003; Hodges et al. 2010; Middlebrooke et al. 2006; Wang 2005). Findings from these studies are inconsistent, with an apparent intensity dependent effect of aerobic exercise on microvascular structure.

Disturbances in microvascular function have an important role in the pathophysiology of type 2 diabetes and may also explain part of the excess cardiovascular disease risk in type 2 diabetes (Tooke 1995). The retinal microvasculature, accessible by direct non-invasive visualization, has recently garnered interest as a site for assessing the systemic microcirculation *in vivo* (Liew et al. 2008c). Significant associations are reported between changes in the retinal microvasculature and both clinical and subclinical cardiovascular (Wang et al. 2006a) and metabolic diseases (Nguyen and Wong 2006). Specifically, changes in retinal arteriolar and venular microvascular calibre have been associated with both macro and microvascular complications in type 2 diabetics (Klein et al. 2007).

Few studies have examined the effect of physical activity on retinal microvascular changes. Wider venular calibre has been associated with lower levels of physical activity (Anuradha et al. 2011b; Anuradha et al. 2011c; Tikellis et al. 2010) and sedentary behaviour in men, but not women (Anuradha et al. 2011a). However, these studies examined only mild levels of physical activity and inactivity (via self-reported

questionnaires) and used a cross-sectional design. Thus, it is unknown whether moderate-intensity exercise has longitudinal beneficial effects on the retinal vasculature.

No studies have yet examined the effects of repeated bouts of moderate-intensity exercise on retinal microvascular morphology. The aim of the present study was to investigate the effect of 12-weeks of supervised, moderate-intensity walking exercise on retinal vascular morphology, including vessel calibre and vascular fractal dimension in women 65-74 years of age with type 2 diabetes.

3.2 Patients and Methods

3.2.1 Subjects and Recruitment Strategies

Fifteen women with type 2 diabetes, aged 65-74 years participated. Subjects were recruited from local family medical clinics, and after media advertisements and mail-outs by Diabetes Australia Queensland. Recruitment was difficult from this age group. Approximately 5,000 flyers were distributed to local GP clinics, diabetes educators, hospitals and pharmacies. Newspaper and radio advertisements were run weekly over a 6-month period. From patient databases and all contacts, approximately 500 potential subjects meeting all eligibility criteria were contacted via mail with help from Diabetes Australia Queensland. After 18 months of extensive recruitment, only 72 persons (14% of those invited) expressed interest in participating, of whom 32 withdrew because of the time commitment needed or because of unwillingness to exercise. After excluding those who did not meet our inclusion criteria, there was a cohort of 16 persons, with only 15 persons completing all repeated testing periods (wk -6, 0, 6, 12) and exercise-training sessions.

Potential subjects were initially screened by telephone interview using a standardized set of questions to determine suitability to enter a formal health screening. The following inclusion criteria were used: (i) clinical diagnosis of type 2 diabetes for at least 12 months, (ii) not currently using exogenous insulin (iii) non-smoking, (iv) normal age-relative physical examination, (v) no significant abnormalities in heart rate and rhythm during a graded treadmill exercise test, (vi) no severe musculoskeletal disability, and (vii)

not currently taking medications known to directly interfere with exercise responses. All subjects provided written consent to participate. Each subject's cognitive capacity to give consent was determined using the Mini-Mental State Examination (Folstein et al. 1975) and assessed by an independent observer. Subjects then entered a formal health-screening phase that included anthropometry and pulmonary function testing. Height was measured (in millimetres) using a wall-mounted stadiometer (Harpenden, Holtain Ltd, Crosswell, UK). Body mass was measured using a calibrated digital scale (CH-150k, A & D Pty Ltd, Thebarton, Australia) and waist and hip circumference were measured. Pulmonary function was assessed (forced vital capacity, forced expiratory volume in 1 second, and forced expiratory volume in 1 second/forced vital capacity ratio) using a calibrated spirometry system (Ultima CPX, Medical Graphics Corporation, St Paul, USA). Supine and standing 12-lead ECG (Cardio Perfect, Welch Allyn Inc., Skaneateles Falls, USA) and blood pressure were measured at rest.

After an overnight fast, venous blood samples were drawn (between 0700 – 0830 hours) at an accredited pathology laboratory for glucose, insulin, haemoglobin A_{1c} (HbA_{1c}), low- and high-density lipoprotein cholesterol, triglycerides and homocysteine measurements (**Appendix E**). Subjects were then asked to visit their family physician with the health-screening results and details of the experimental procedures for an opinion about their suitability to participate. Bond University Human Research Ethics Committee and Griffith University Human Ethics Committee reviewed and approved the experimental protocol (RO-745).

3.2.2 Determination of Peak Oxygen Uptake and Gas-Exchange Threshold

Prior to determining peak oxygen uptake, all subjects were thoroughly familiarized with all intended procedures and equipment. Subjects then completed an incremental exercise test to volitional fatigue on a motor driven treadmill (‘Valiant’; Lode B.V., Groningen, Netherlands) to determine VO_2peak, TE and T_{ge}. Before the first exercise testing session, subjects were familiarized with treadmill walking at various speeds (2.0 – 6.0 km h^{-1}) and treadmill grades (0 – 10%). Each subject’s preferred walking speed was determined (at 1% grade). This was used as the treadmill speed during all subsequent exercise tests and

training sessions. The incremental exercise test consisted of a 4-min warm-up at 3.0 $km \cdot h^{-1}$ and 1% grade, followed by a speed increase every minute until the previously determined self-selected speed was attained. Treadmill grade was then increased by 2% every minute until volitional fatigue or clinically significant signs or symptoms precluded further exercise. Cardiac rate and rhythm was monitored continuously using a 12 lead ECG and brachial artery blood pressure was measured by auscultation every 3 minutes during the incremental exercise test. VCO_2, VO_2, and V_E BTPS were measured breath-by-breath and averaged every 30 seconds using an open-circuit metabolic measurement system (Ultima CPX, Medical Graphics Corporation, St Paul, USA). The gas analysers and pneumotachograph were calibrated before and after each incremental exercise test using precision reference gases and an independently calibrated 3-L calibration syringe (Hans Rudolph Inc., Kansas City, USA). The average of the two highest consecutive 30-second values measured before volitional fatigue was used to determine peak gas exchange (VO_2, VCO_2) values.

3.2.3 Exercise Training Program

After recruitment, dependent variables were measured 6 weeks before commencing exercise training (acting as a control period for each subject) (wk -6), immediately before starting the exercise training program (wk 0), and after 6- and 12-weeks exercise training. All subjects exercised by walking on a motor driven treadmill for a total of 120 $minute \cdot week^{-1}$, at an intensity equivalent to their individual T_{ge}. Before commencing exercise training (wk 0), subjects were again familiarized with walking on a motor-driven treadmill. Subjects completed a 3-minute warm-up and a 3-minute cool-down at $3km \cdot hr^{-1}$ and 1% grade for all exercise training sessions. Before each exercise training session, subjects were fitted with a 5-lead ECG X12+ (Mortara Instrument Inc., Milwaukee, USA) to monitor heart rate and rhythm continuously. Heart rate and blood pressure were recorded every 5 minutes throughout all exercise training sessions.

Exercise training intensity was individually determined during the first training session using breath-by-breath open-circuit spirometry to determine VO_2. While at each subject's self-selected walking speed, treadmill grade was increased 1% every 3 minutes until

steady-state VO_2 matched their pre-determined T_{ge}. Based on maximal exercise test results obtained after 6 weeks of exercise training (wk 0-6), exercise intensity was adjusted to maintain the same relative percent (100%) of T_{ge} for the remaining 6 weeks of exercise training. Subjects were instructed to continue their normal daily activities during both the 6-week control period and 12-week exercise-training program.

3.2.4 Retinal Photography and Measurement of Retinal Vascular Calibre

After pupil dilation, colour static digital fundus photographs (2 fields of each eye, one centred on the optic disk and a second on the macula) were taken using a 45° 6.1 megapixel digital non-mydriatic camera (Nikon D100, Nikon Corporation, Japan). Retinal photographs were taken within 24-48 hours of the last exercise session during the intervention period (wk 6 and 12). Images were graded by the Retinal Vascular Imaging Centre (RetVIC), Centre for Eye Research, Melbourne, Australia for measurements of retinal vascular morphology.

RVC was measured by trained graders masked to participant characteristics using a computer-based program (Retinal Analysis, University of Wisconsin, Madison, WI, USA) (Wong et al. 2004b). Briefly, the diameter of all arterioles and venules within a concentric zone corresponding to a half- to one-disc diameter away from the optic disc margin were measured. Diameters of the widest six arterioles and venules were summarized as central retinal artery equivalent (CRAE) and central retinal vein equivalent (CRVE). CRAE and CRVE represent the estimated average calibre of the central retinal artery and vein, respectively (Wong et al. 2004b). The AVR was calculated as CRAE divided by CRVE. Retinal images from the right eye were analysed. Intra- and inter-grader reproducibility of retinal vascular measurements are previously reported, with the intra-class correlation coefficients between 0.78 and 0.99 (Wong et al. 2004b). The intra-class correlation for the present study, based on re-grading of 20 randomly chosen photographs was confirmed and ranged from 0.79 to 0.98.

3.2.5 Measurement of Retinal Fractal Dimension

Fractal analysis was completed by trained graders, masked to participant characteristics, using a computer-based program (International Retinal Imaging Software [IRIS],

National University of Singapore, University of Melbourne, University of Sydney; Patent pending), which measured D_f (Liew et al. 2008b). Retinal vascular D_f of each image was calculated within a 3.5-disc radii pre-defined circular area centred on the optic disk. Retinal vessels within this region were automatically traced by the software and subsequently examined by graders to identify and erase artefacts (e.g., peripapillary atrophy, choroidal vessels, and pigment abnormalities) that could be mistaken by the software as retinal vessels. Fractal analysis was then performed by the IRIS software and D_f calculated using a pre-established box counting approach (Liew et al. 2008b). Reliability of the IRIS measurements was high, with intra-grader intra-class correlation coefficients ranging from 0.93 to 0.94.

3.2.6 Statistical Analysis

Results are reported as mean ± standard error unless otherwise stated. Data were analysed after adjusting for mean arterial blood pressure (MAP) using a repeated measures analysis of variance with exercise group as the between-subject variable, and weeks (wk 0, 6, 12) as the within-subject variable. All statistical differences were accepted at the $p < 0.05$ level. SPSS (SPSS Inc, Release 17.0, USA) was used.

3.3 Results

No significant differences were detected in any dependent variable before or after the 6-week (wk -6 to 0) intervention-free control period (**Table 1**).

After 6 and 12-weeks of exercise training, there were no significant changes in body mass, BMI or waist-to-hip ratio. However, both systolic ($p = 0.014$) (**Figure 1**) and diastolic ($p = 0.032$) (**Figure 1**) blood pressure were significantly reduced after 12-weeks of exercise training, as was HDL ($p = 0.035$). No significant changes were found in other blood or lipid profile measurements after 12-weeks of exercise training.

Twelve weeks of exercise training at an intensity equivalent to T_{ge} (72-74 % VO_2peak) resulted in significant increases in time to exhaustion ($p < 0.001$), VO_2peak ($p = 0.016$),

VO_2 at T_{ge} ($p = 0.030$), and heart rate at T_{ge} ($p = 0.033$), VO_2peak relative to body mass ($p = 0.026$) (**Figure 2**), and peak respiratory exchange ratio ($p = 0.040$).

Using MAP as a covariate, no significant changes were found in CRAE ($p = 0.464$), CRVE ($p = 0.519$), or AVR ($p = 0.879$) after 6 and 12-weeks exercise training (**Table 2, Figure 3**). Retinal fractal dimension (D_f) ($p = 0.284$) was also unchanged after 6- and 12-weeks of exercise training (**Table 2, Figure 3**).

3.4 Discussion

Our study found that 12-weeks of moderate-intensity exercise training (120 minutes per week at an intensity equivalent to individual T_{ge}) did not significantly alter RVC or retinal D_f in women aged 65-74 years with type 2 diabetes. Although RVC may have changed immediately after each exercise training session due to increases in blood flow associated with exercise, any such changes in the retinal microvasculature must have been short lived. Twelve weeks of exercise training, did result in significant increases in HDL, exercise time to exhaustion, absolute and relative VO_2peak, T_{ge} and significant decreases in systolic and diastolic blood pressure.

Subjects in the present study had similar anthropometric characteristics and responses to exercise as those reported in other studies of older women with type 2 diabetes (McGavock et al. 2004). Despite exercising for 120 minutes/week, 12-weeks of moderate-intensity exercise training did not decrease BMI, body mass, or waist-to-hip ratio, consistent with previous studies (Middlebrooke et al. 2006). The lack of improvement in BMI, waist-to-hip ratio and body mass may reflect the short duration of the exercise training program, and any decreases in fat mass could be offset by initial increases in muscle mass. In agreement with our study, moderate intensity, aerobic exercise has been effective in improving VO_2peak and time to exhaustion (McGavock et al. 2004). In a study of post-menopausal women, aged 51-67 years with type 2 diabetes, 10 weeks of cycle ergometry, 3 times / week for 55 minutes per session at 75% of heart rate reserve (HRR) resulted in a 15% increase in VO_2peak (McGavock et al. 2004). In our study, the increase in VO_2peak was approximately 9%, possibly explained by the shorter weekly exercise duration (120 vs. 165 minutes) and the older age of the women.

Studies available for comparison have examined changes in retinal vascular calibre associated with diseases involving macrovascular endothelial dysfunction, including coronary heart disease (Wong et al. 2002a), hypertension (Wong et al. 2005b), heart failure (Wong et al. 2005a), and type 1 and type 2 diabetes (Nguyen et al. 2008; Wong et al. 2002b). While evidence supporting the effectiveness of exercise training in improving macrovascular function is well documented and consistent (Haddock et al. 1998; Woolf et al. 2008), the evidence is limited and inconsistent with regards to exercise-related changes in microvascular function (Black et al. 2008; Goto et al. 2003; Hamdy et al. 2003; Hodges et al. 2010; Middlebrooke et al. 2006; Wang 2005).

Prior to our study, most research examining the effects of exercise training on human microvasculature has focused on cutaneous microvessels (Black et al. 2008; Goto et al. 2003; Hamdy et al. 2003; Hodges et al. 2010; Middlebrooke et al. 2006; Wang 2005). This vascular bed, like retinal microvessels (Wong et al. 2006), has been shown to reflect generalized microvascular function (Holowatz et al. 2008) and may function as a measure of pre-clinical microvascular disease. In individuals with microvascular dysfunction associated with type 2 diabetes, effects of exercise training on cutaneous microvascular function have been examined (Black et al. 2008; Goto et al. 2003; Hamdy et al. 2003; Hodges et al. 2010; Middlebrooke et al. 2006; Wang 2005). Among individuals with type 2 diabetes (Middlebrooke et al. 2006), or obese individuals with insulin-resistance syndrome (Hamdy et al. 2003), regular exercise did not significantly improve microvascular function as measured by cutaneous reactivity. Furthermore, after a 6-month exercise-training program (at 70-80% of maximal heart rate) for people with type 2 diabetes (Middlebrooke et al. 2006), there was no significant improvement in microvascular function or in measures of “aerobic fitness”, raising concern about the effectiveness of the selected exercise training stimulus. Our study, together with work by Hamdy et al. (Hamdy et al. 2003), report no significant improvements in microvascular structure with exercise training, despite increases in VO_2peak and related measures. Hamdy et al. (Hamdy et al. 2003) reported a significant increase in macrovascular endothelial function, but no cutaneous microvascular function change following 6 months

of weight reduction and exercise training in obese persons aged 30-65 years with insulin-resistance syndrome.

Improvements in microvascular function with aerobic exercise have been reported (Black et al. 2008; Wang 2005), but with an apparent intensity dependent effect. Moderate-intensity (50% VO_2peak) aerobic exercise, as opposed to low (< 25% VO_2peak) and high intensity (> 75% VO_2peak) aerobic exercise, has been shown to improve cutaneous microvascular function in healthy adults after 8 weeks of exercise training (Wang 2005). Moderate-intensity exercise training for 24 weeks was found to prevent the decline in microvascular nitric oxide-mediated vasodilator function in older, healthy individuals (Black et al. 2008). Differences in exercise intensity reported in other studies (Black et al. 2008; Wang 2005) and in our study could explain why no significant changes were observed in the retinal microvasculature.

In the present study, exercise training intensity averaged approximately 72% VO_2peak, bordering on high-intensity exercise. It has been suggested that relatively "mild intensity" (< 25% VO_2peak) and "high intensity" (> 75% VO_2peak) aerobic exercise may not elicit changes in microvascular function (Goto et al. 2003), possibly explaining in part the results of our study. Goto et al. (Goto et al. 2003) found that 12-weeks of "mild intensity" and "high intensity" aerobic exercise did not significantly improve microvascular endothelial function in young men, while individuals exercising at "moderate" intensity (50% VO_2peak) showed significant improvements in microvascular function. The authors suggested that low-intensity exercise may be an insufficient stimulus for improvement, while high-intensity exercise may impair endothelium-dependent vasodilation by reducing nitric oxide bio-availability through decreased antioxidant levels and increases in reactive oxygen species (Goto et al. 2003). Subjects in our study could have been exercising at an intensity (72% VO_2peak) that impaired endothelium-dependent vasodilation, thereby preventing improvements in retinal microvascular structure.

A recent study strengthened the results of Goto et al (Goto et al. 2003). Hodges et al. (Hodges et al. 2010) found that in post-menopausal women, 48 weeks mild-intensity (<

45% HRR) exercise training was associated with improved cutaneous vascular function. Significant increases in cutaneous vascular conductance occurred during the first 24 weeks of mild-intensity exercise. After this period, increases in the intensity of exercise training to 60 and 75 % of HRR did not elicit further significant improvements in cutaneous vascular function, despite increases in VO_2peak. That exercise intensity can differentially affect microvascular function has important implications for people who select exercise as a therapeutic intervention to manage chronic diseases, such as type 2 diabetes.

Few studies have examined potential associations between retinal vascular morphology and physical activity (Anuradha et al. 2011a; Anuradha et al. 2011b; Anuradha et al. 2011c; Tikellis et al. 2010). Higher levels of physical activity during sport and work were significantly associated with wider venular calibre in the ARIC Study (Tikellis et al. 2010). In the Multi-Ethnic Study of Atherosclerosis, lower levels of physical activity were associated with wider retinal venular calibre in non-Hispanic whites and Hispanics, but not in Blacks or Chinese (Anuradha et al. 2011b). Anuradha et al. (Anuradha et al. 2011a) found no significant relationship between physical activity (self-reported questionnaire) and retinal vascular calibre. However, in men, but not women, sedentary behaviour (as measured by television viewing time) was associated with wider venular calibre (Anuradha et al. 2011a). While small cross-sectional influences of mild physical activity on retinal vascular structure have been reported, longitudinal studies using exercise of higher intensity would be needed for direct comparison to the present study.

Many subjects in our study were taking antihypertensive and/or lipid-lowering medications, which could have improved vascular function to a point where 12-weeks of exercise training may have provided no further benefit. More likely, 12-weeks exercise training may have been too short a duration to demonstrate significant improvements in retinal vascular morphology. The autoregulation of hemodynamics in the retinal vasculature, particularly in arterioles, may have also contributed to the non-significant findings (Pournaras et al. 2008).

Subject recruitment posed difficulty because of the significant time requirements of the exercise training program and the use of a repeated measure design. Most in our cohort were relatively healthy, active and had reasonable diabetic control, so that further significant improvements in microvascular morphology with exercise could be more difficult in these subjects than in others.

In conclusion, the present study suggests that in women aged 65-74 years with type 2 diabetes, 12-weeks moderate-intensity exercise training (walking) did not significantly affect retinal microvascular structure reflected by measures of retinal vascular calibre and fractal dimension. Further investigation with a longer duration of exercise and a range of exercise intensities may help to determine the effectiveness of repeated bouts of exercise training on the retinal microcirculation.

Table 1. Clinical characteristics of participating subjects before (wk -6) and after (wk 0) 6-week intervention-free control period. Mean ± SEM.

	Week -6 (n=15)	Week 0 (n=15)
Age (years)	68.9±0.7	68.9±0.7
Body Mass (kg)	77.1±3.5	76.5±3.6
BMI ($kg \cdot m^{-2}$)	30.1±1.3	29.9±1.3
Waist/Hip Ratio	0.86±0.02	0.85±0.02
Blood Pressure (mmHg)		
SBP_{rest}	133±3	127±2
DBP_{rest}	76±2	74±2
Blood Profile		
Homocysteine ($\mu mol \cdot L^{-1}$)	12.3±1.3	12.1±1.3
Glucose ($mmol \cdot L^{-1}$)	7.3±0.4	7.3±0.5
HbA_{1C} (%)	6.6±0.2	6.6±0.2
Insulin ($mU \cdot L^{-1}$)	11.1±2.2	10.9±2.2
Lipid Profiles ($mmol \cdot L^{-1}$)		
Total Cholesterol	3.4±0.2	3.3±0.2
Triglycerides	1.4±0.2	1.3±0.1
HDL	1.4±0.1	1.4±0.1
LDL	2.4±0.2	2.4±0.2
Exercise Responses		
TE (min)	13.8±0.6	13.9±0.5
VO_2peak ($L \cdot min^{-1}$)	1.52±0.04	1.48±0.05
VO_2peak ($mL \cdot kg^{-1} \cdot min^{-1}$)	20.2±0.8	19.7±1
Peak HR ($b \cdot min^{-1}$)	144±3	141±4
V_E peak ($L \cdot min^{-1}$)	53.1±1.9	51.9±2.4
VO_2T_{ge} ($L \cdot min^{-1}$)	1.1±0.2	1.0±0.2
Peak RER	1.09±0.02	1.10±0.02

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HbA_{1c}, glycated haemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TE, time to exhaustion; VO_2peak, peak oxygen uptake; HR, heart rate; V_E, ventilation; VO_2 T_{ge}, oxygen uptake at gas-exchange threshold; RER, respiratory exchange ratio.

Table 2. Retinal vessel measurements of participating subjects by week (-6,0,6,12). Mean ± SEM.

	Week -6 (n=15)	Week 0 (n=15)	Week 6 (n=15)	Week 12 (n=15)
CRAE (μm)	148±6	149±6	148±6	146±7
CVRE (μm)	213±5	215±4	216±6	216±6
AVR	0.70±0.01	0.70±0.02	0.70±0.02	0.70±0.02
Fractal Dimension (D_f)	1.46±0.01	1.46±0.01	1.45±0.01	1.45±0.01

CRAE, central retinal artery equivalent; CRVE, central retinal venular equivalent; AVR, artery-to-venule ratio.

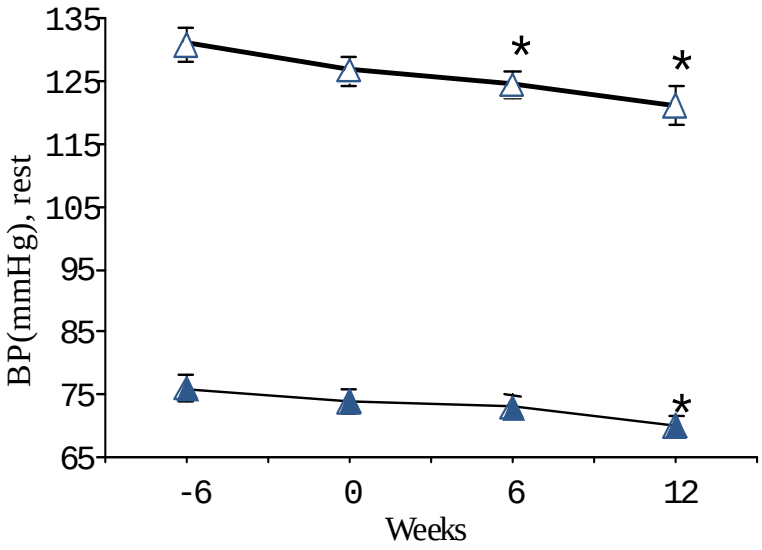


Figure 1. Changes in systolic blood pressure (△) and diastolic blood pressure (▲) during 6-week control period (wk -6 to wk 0), and after 6 and 12-weeks of exercise training (wk 0 to wk 6 to wk 12). Mean ± SEM. *, $p < 0.05$, significantly different to pre-training (wk 0).

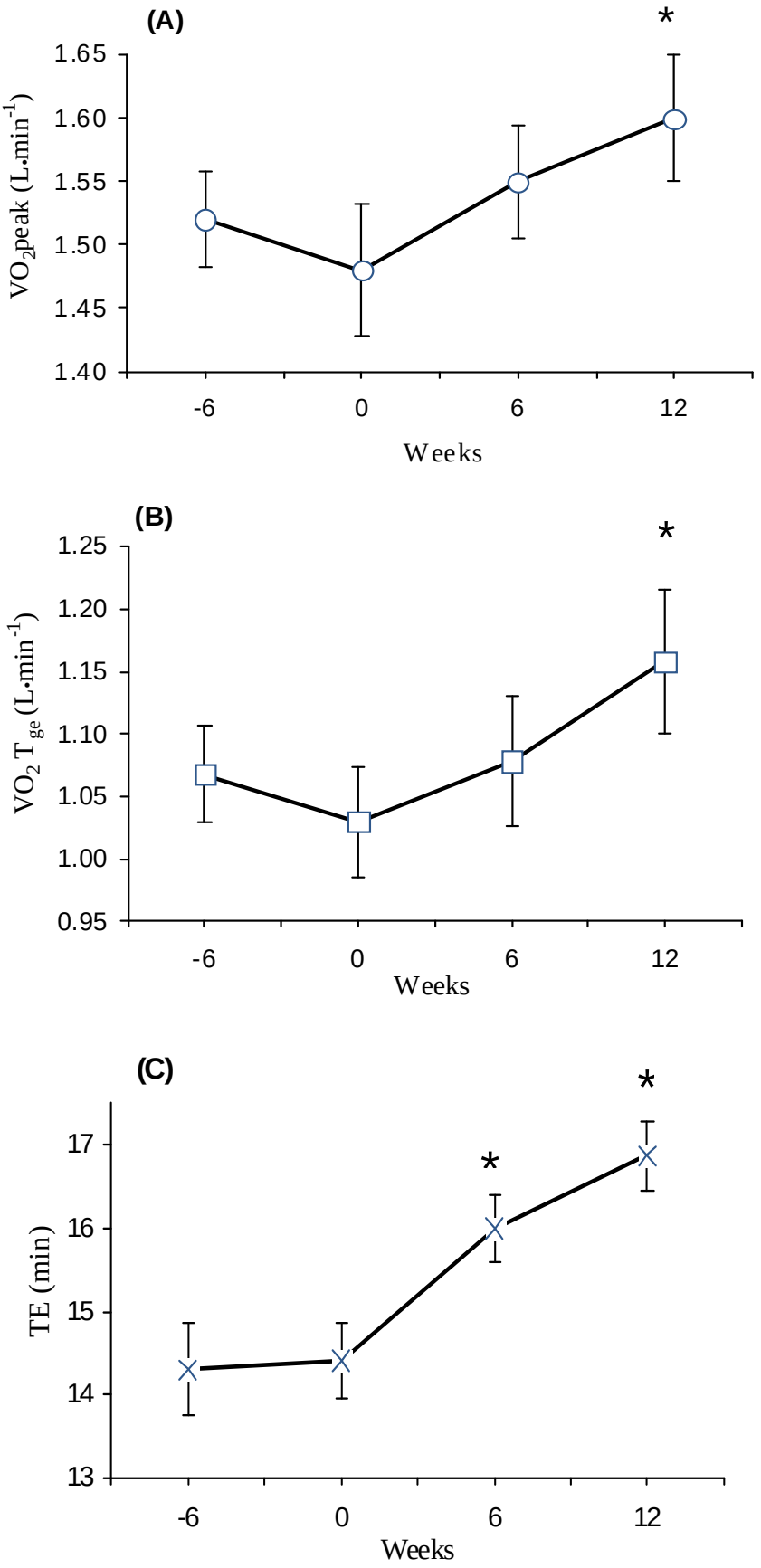


Figure 2. **(A)** Changes in peak VO_2 (○), **(B)** VO_2 at T_{ge} (□), **(C)** time to exhaustion (TE) (X) during 6-week control period (wk -6 to wk 0), and after 6 and 12-weeks of exercise training (wk 0 to wk 6 to wk 12). Mean ± SEM. *, $p < 0.05$, significantly different to pre-training (wk 0).

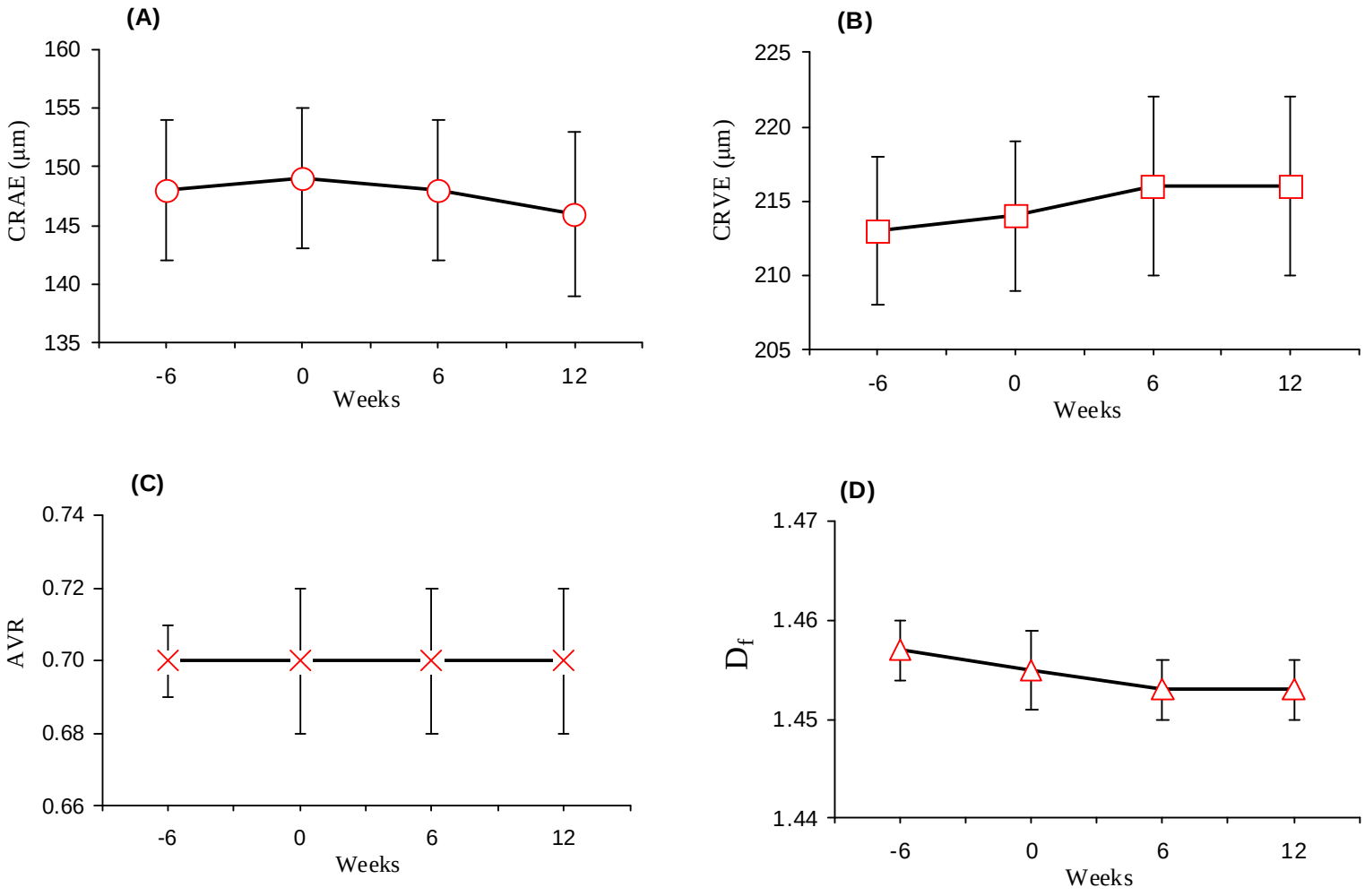


Figure 3. **(A)** Changes in CRAE (○), **(B)** CRVE (□), **(C)** AVR (X), and **(D)** D_f (△) during 6-week control period (wk -6 to wk 0), and after 6 and 12-weeks of exercise training (wk 0 to wk 6 to wk 12). Mean ± SEM.

CHAPTER 4 – *Study Three*

Effect of Exercise Training on Asymmetric Dimethylarginine Concentration in Women Aged 65-74 Yr with Type 2 Diabetes

4.1 Introduction

Type 2 diabetes is associated with a two-to-three fold higher incidence of macrovascular atherosclerotic disease compared to non-diabetic individuals (Kannel and McGee 1979). The increased incidence of microvascular atherosclerotic disease cannot be fully explained by the presence of conventional risk factors such as hypertension, obesity, and dislypidaemia (Yamagishi and Imaizumi 2005), that often accompany diabetes. Decreased NO bioavailability, a key modulator of endothelial function, may contribute to the development of the microvascular complications associated with type 2 diabetes (Schalkwijk and Stehouwer 2005), which is in part modulated by ADMA (Kawata et al. 2005; Lin et al. 2002).

ADMA is an endogenous, competitive inhibitor of NO synthase and a known independent cardiovascular risk factor (Vallance et al. 1992a; Vallance and Leiper 2004). Plasma ADMA concentrations have been shown to be elevated in individuals with increased cardiovascular risk (Boger et al. 1998; Schnabel et al. 2005; Surdacki et al. 1999; Vallance et al. 1992b), older individuals (Miyazaki et al. 1999), and in patients with type 2 diabetes (Abbasi et al. 2001; Kawata et al. 2005; Lin et al. 2002). Elevated plasma ADMA concentrations are thought to play a role in the endothelial dysfunction and associated vascular complications seen in individuals with type 2 diabetes (de Jager et al. 2006; Malecki et al. 2007; Yamagishi et al. 2008). While regular physical exercise has been shown to improve endothelial function in individuals with type 2 diabetes (Maiorana et al. 2001; Okada et al. 2010), the pathophysiological basis for these exercise-induced improvements remains unclear.

Regular physical exercise has been shown to reduce circulating levels of ADMA in patients with increased risk of coronary heart disease (Richter et al. 2005), in patients with metabolic syndrome (Gomes et al. 2008) and type 1 diabetes mellitus (Mittermayer et al. 2005). To date, no study has examined the effects of repeated bouts of moderate-intensity exercise on ADMA concentration in individuals with type 2 diabetes. Therefore, the aim of the present study was to determine the effects of 12-weeks of moderate-intensity exercise on plasma ADMA in women 65-74 years of age with type 2 diabetes.

4.2 Methods

4.2.1 Participants and Recruitment Strategies

Fifteen women with type 2 diabetes, aged 65-74 years participated. Participants were recruited from local GP clinics, media advertisements and mail-outs by Diabetes Australia Queensland. Recruitment was difficult from this age group. Approximately 5,000 flyers were distributed to local GP clinics, diabetes educators, hospitals and pharmacies. Newspaper and radio advertisements were run weekly over a 6-month period. From patient databases and all contacts, approximately 500 potential participants meeting all eligibility criteria were contacted via mail with help from Diabetes Australia Queensland. After 18 months of extensive recruitment, only 72 persons (14% of those invited) expressed interest in participating, of which 32 withdrew because of the time commitment needed or because of their unwillingness to exercise. After excluding those who did not meet our inclusion criteria, there was a cohort of 16 persons; with only 15 persons completing all repeated testing periods (wk -6, 0, 6, 12) and all exercise-training sessions.

Potential participants were initially screened by telephone interview using a standardized set of questions to determine suitability to enter a formal health screening. The following inclusion criteria were used: (i) clinical diagnosis of type 2 diabetes for at least 12 months, (ii) not currently using exogenous insulin (iii) non-smoking, (iv) normal age-relative physical examination, (v) no significant abnormalities in heart rate and rhythm during a graded treadmill exercise test, (vi) no severe musculoskeletal disability and (vii) not currently taking medications known to directly interfere with exercise responses. All participants provided written consent to participate. Each subject's cognitive capacity to give consent was determined using the Mini-Mental State Examination (Folstein et al. 1975) and assessed by an independent observer. Participants then entered a formal health-screening phase that included anthropometry and pulmonary function testing. Height was measured (in millimetres) using a wall-mounted stadiometer (Harpenden, Holtain Ltd, Crosswell, UK). Body mass was measured using a calibrated digital scale (CH-150k, A & D Pty Ltd, Thebarton, Australia) and waist and hip circumference were measured. Pulmonary function was assessed (forced vital capacity, forced expiratory volume in 1

second, and forced expiratory volume in 1 second/forced vital capacity ratio) using a calibrated spirometry system (Ultima CPX, Medical Graphics Corporation, St Paul, USA). Supine and standing 12-lead ECG (Cardio Perfect, Welch Allyn Inc., Skaneateles Falls, USA) and blood pressure were measured at rest.

After an overnight fast, venous blood samples were drawn (between 0700 – 0830 hours) at an accredited pathology laboratory for measurements of glucose, insulin, haemoglobin A_{1c} (HbA_{1c}), and homocysteine concentrations (**Appendix E**). Participants were then asked to visit their family physician with the health-screening results and details of the experimental procedures for an opinion about their suitability to participate in the exercise training program. Bond University Human Research Ethics Committee and Griffith University Human Ethics Committee reviewed and approved the experimental protocol (RO-745).

4.2.2 Determination of Peak Oxygen Uptake and Gas-Exchange Threshold

Prior to determining peak oxygen uptake, all participants were thoroughly familiarized with all procedures and equipment. Participants then completed an incremental exercise test to volitional fatigue on a motor driven treadmill ('Valiant'; Lode B.V., Groningen, Netherlands) to determine VO_2peak, TE and T_{ge}. Before the first exercise testing session, participants were familiarized with treadmill walking at various speeds (2.0 – 6.0 km h^{-1}) and treadmill grades (0 – 10%). Each subject's preferred walking speed was determined (at 1% grade). Preferred walking speed was used as the treadmill speed during all subsequent exercise tests and exercise training sessions. The incremental exercise test consisted of a 4-min warm-up at 3.0 km·h^{-1} and 1% grade, followed by a speed increase every minute until the previously determined preferred walking speed was attained. Treadmill grade was then increased by 2% every minute until volitional fatigue or clinically significant signs or symptoms precluded further exercise. Cardiac rate and rhythm was monitored continuously using a 12 lead ECG and brachial artery blood pressure was measured by auscultation every 3 minutes during the incremental exercise test. VCO_2, VO_2, and V_E BTPS were measured breath-by-breath and averaged every 30 seconds using an open-circuit metabolic measurement system (Ultima CPX, Medical

Graphics Corporation, St Paul, USA). The gas analysers and pneumotachograph were calibrated before and after each incremental exercise test using precision reference gases and an independently calibrated 3-L calibration syringe (Hans Rudolph Inc., Kansas City, USA). The average of the two highest consecutive 30-second values measured before volitional fatigue was used to determine peak gas exchange (VO_2, VCO_2) values. T_{ge} was determined using the simplified V-slope method (Schneider et al. 1993).

4.2.3 Exercise Training Program

After recruitment, dependent variables were measured 6 weeks before commencing exercise training (acting as a control period for each subject) (wk -6), immediately before starting the exercise training program (wk 0), and after 6 and 12-weeks of exercise training. All participants exercised by walking on a motor driven treadmill for a total of 120 minute·week^{-1}, at an intensity equivalent to their individual T_{ge}. Before commencing exercise training (wk 0), participants were again familiarized with walking on a motor-driven treadmill. Participants completed a 3-minute warm-up and a 3-minute cool-down at 3km·hr^{-1} and 1% grade for all exercise training sessions. Before each exercise training session, participants were fitted with a 5-lead ECG X12+ (Mortara Instrument Inc., Milwaukee, USA) to continuously monitor heart rate and rhythm. Heart rate and blood pressure were recorded every 5 minutes throughout all exercise training sessions.

Exercise training intensity was individually determined during the first exercise training session using breath-by-breath open-circuit spirometry, to determine VO_2. While at each subject's preferred walking speed, treadmill grade was increased 1% every 3 minutes until steady-state VO_2 matched their pre-determined T_{ge}. Based on maximal exercise test results obtained after 6 weeks of exercise training (wk 0-6), exercise intensity was adjusted to maintain the same relative percent (100%) of T_{ge} for the remaining 6 weeks exercise training. Participants were instructed to continue their normal daily activities during both the 6-week control period and 12-week exercise-training program.

4.2.4 Determination of Asymmetric Dimethylarginine

Blood was collected from a prominent antecubital vein into ethylenediaminetetraacetic acid (EDTA) blood collection tubes, which were immediately placed on a tube roller. Plasma was separated from whole blood within 30 min of collection and subsequently stored at -80 °C until analysed. ADMA concentrations were assayed (in fasting plasma) using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Immundiagnostik AG, Bensheim, Germany). A Fluido microplate strip-washer (Anthos Labtec Instruments, Eugendorf, Austria) and Anthos 2020 absorbance reader (Anthos Labtec Instruments, Eugendorf, Austria) were utilized during all assays. The ELISA kit reported a sensitivity of 0.05 $\mu mol \cdot L^{-1}$. The intra-assay coefficient of variation ranged from 5.3-7.7%. Standards and controls provided with the kits were used in measurements.

4.2.5 Statistical Analysis

Results are reported as mean ± standard error unless otherwise stated. Data were analysed using a mixed design analysis of variance (ANOVA) with exercise group as the between-subject variable, and weeks (wk 0, 6, 12) as the within-subject variable. All statistical differences were accepted at the $p < 0.05$ level. SPSS (SPSS Inc, Release 17.0, USA) was used for all statistical analyses.

4.3 Results

Of the fifteen individuals who started the exercise training program, fourteen individuals completed all repeated testing periods, blood draws, and exercise training sessions. No significant differences were found in any dependent variables before or after the 6-week intervention-free control period (wk -6 to 0) (**Table 1**).

After 6 and 12-weeks of exercise training, there were no significant changes in body mass, BMI or waist-to-hip ratio. However, both systolic and diastolic blood pressure was significantly reduced after 12-weeks of exercise training ($p > 0.05$). Plasma ADMA concentration was found to be significantly lower after 12-weeks exercise training from baseline measurements (wk 0) ($p < 0.05$; **Table 1; Figure 1**). No significant changes

were found in other blood or lipid profile measurements after 12-weeks of exercise training ($p > 0.05$) (**Table 1**).

Twelve weeks of exercise training at an intensity equivalent to Tge (72-74 % VO_2peak) resulted in significant increases in time to exhaustion, absolute and relative VO_2peak, and VO_2 at T_{ge} ($p < 0.05$) (**Table 1**).

4.4 Discussion and Conclusions

Our study found that 12-weeks of moderate-intensity exercise training (120 minutes per week at an intensity equivalent to individual T_{ge}) significantly lowered plasma concentration of circulating ADMA in women aged 65-74 years with type 2 diabetes. To our knowledge, this is the first study showing clinical evidence for exercise as a therapeutic intervention to lower ADMA concentration in older women with type 2 diabetes.

Participants in the present study had plasma ADMA levels consistent with those found in other studies of post-menopausal women (Sydow et al. 2010). In agreement with the present study, exercise-induced decreases in systemic ADMA concentration have been previously reported in different populations (Hanssen et al. 2011; Mittermayer et al. 2005; Richter et al. 2005; Schlager et al. 2011; Sydow et al. 2010; Tsarouhas et al. 2011). Moderate-intensity, supervised exercise has shown to decrease plasma ADMA in participants with peripheral arterial disease (Schlager et al. 2011), chronic heart failure (Tsarouhas et al. 2011), obesity (Hanssen et al. 2011), elevated cardiovascular risk (Richter et al. 2005), and type 1 diabetes (Mittermayer et al. 2005). Exercise training has also been shown to be ineffective in decreasing plasma ADMA (Niebauer et al. 2005). Plasma ADMA concentration was unaffected by 2 months of unsupervised, home-based exercise training in heart failure patients (Niebauer et al. 2005). ADMA concentration was similar between patients and control participants, and normal ADMA concentrations might not have been further lowered by exercise. Plasma ADMA concentration was also found to be unaffected by 16 weeks of supervised exercise training in another cohort of heart failure patients (Seljeflot et al. 2011). However, the exercise dose may have been

insufficient to elicit a decrease in ADMA concentration as the exercise mode (dancing) was performed in a group environment and music speed was used to moderate intensity according self-reported rating of perceived exertion (Seljeflot et al. 2011). Unlike the present study, exercise stimulus was not individualized nor linked to a physiological marker such as T_{ge}.

In the present study, twelve weeks of moderate-intensity exercise training resulted in significant improvements in multiple measures of physiological functional capacity, including TE, VO_2peak, and VO_2 at T_{ge}. These improvements were accompanied by significant decreases in both systolic and diastolic blood pressure following twelve weeks of exercise. The exercise-induced reductions in ADMA plasma concentration has been reported elsewhere (Hanssen et al. 2011; Mittermayer et al. 2005; Richter et al. 2005; Schlager et al. 2011; Sydow et al. 2010; Tsarouhas et al. 2011), the mechanisms behind this process are not clear. ADMA's cardiovascular effects include vasoconstriction and increased blood pressure (Achan et al. 2003; Kielstein et al. 2004) and may explain, in part, the significant reduction in BP seen in the present study after 12-weeks of exercise training. This argument may be strengthened by research suggesting that exercise-induced increases in NO bioavailability may be due, at least in part, to decreases in ADMA concentration (Gomes et al. 2008).

It has been hypothesized that regular exercise decreases ADMA concentration by upregulating dimethylarginine dimethylaminohydrolase 1 (DDAH-1), an enzyme involved in ADMA decomposition (Mittermayer et al. 2005). Recently, decreased ADMA concentration following exercise training has been shown to be accompanied by enhanced DDAH-1 mRNA gene-expression (Hanssen et al. 2011). Upregulation of DDAH-1 and subsequent decreased ADMA concentration through regular exercise may be influenced by antioxidant status. Recent work has suggested that aging-associated oxidant/antioxidant imbalance promotes the formation cardiovascular risk factors, including ADMA, via increases in systemic oxidative stress in elderly individuals (Fabian et al. 2011). Unfortunately, no measure of oxidative stress was measured in the present study and these findings cannot be confirmed.

The significant decrease in circulating plasma ADMA concentration in the present study was not accompanied by significant improvements in conventional markers of CVD risk factors, including homocysteine concentration. The reduction in plasma ADMA concentration may be independent of homocysteine and may act as a sensitive marker of CVD risk in medically well-managed patients who may not show more conventional signs of CVD risk.

Our study's limitations include: extrapolation of the results beyond the cohort of participants in the present study. While subject recruitment proved to be very difficult due to the significant time requirements of the exercise training program and the use of a repeated measure design, the modest sample size used in our study needs to be acknowledged as a potential limitation, but mitigated by the present experimental design.

In conclusion, 12-weeks moderate-intensity exercise training (walking) can significantly reduce circulating plasma ADMA concentration and systolic and diastolic blood pressure in women aged 65-74 years with type 2 diabetes. Our study suggests that the decreased incidence of vascular disease associated with regular exercise in type 2 diabetes may be, in part, explained by decreased circulating plasma ADMA concentration.

Table 1. Clinical characteristics of participating subjects before (wk -6) and after (wk 0) 6-week intervention-free control period and after 6 and 12-weeks of exercise training. Mean ± SEM.

	Week -6 (n=14)	Week 0 (n=14)	Week 6 (n=14)	Week 12 (n=14)
Age (yr)	68.9±0.7	68.9±0.7	68.9±0.7	68.9±0.7
Body Mass (kg)	76.5±3.7	75.9±3.8	76.2±3.9	76.4±3.8
BMI ($kg \cdot m^{-2}$)	30.2±1.4	30.0±1.4	30.1±1.4	30.1±1.4
Waist/Hip Ratio	0.86±0.02	0.85±0.02	0.86±0.02	0.86±0.02
Blood Pressure (mmHg)				
SBP_{rest}	134±3	133±2	130±3	122±3*
DBP_{rest}	76±2	75±2	70±2	69±2*
Blood Profile				
ADMA ($\mu mol \cdot L^{-1}$)	0.68±0.04	0.71±0.02	0.69±0.04	0.62±0.03*
Homocysteine ($\mu mol \cdot L^{-1}$)	12.8±1.8	11.6±1.3	11.6±0.9	11.6±0.8
Glucose ($mmol \cdot L^{-1}$)	7.4±0.5	7.5±0.5	7.0±0.4	7.2±0.4
HbA_{1C} (%)	6.6±0.2	6.6±0.2	6.5±0.2	6.7±0.2
HbA_{1C} ($mmol \cdot mol^{-1}$)	49±2	49±2	48±2	50±2
Insulin ($mU \cdot L^{-1}$)	11.3±2.3	11.0±2.2	12.9±2.6	13.6±3.5
Exercise Responses				
TE (min)	13.7±0.5	13.9±0.4	15.4±0.4*	16.4±0.4*
VO_2peak ($L \cdot min^{-1}$)	1.49±0.03	1.45±0.04	1.53±0.05	1.59±0.05*
VO_2peak ($mL \cdot kg^{-1} \cdot min^{-1}$)	20.0±0.9	19.6±1.1	20.5±0.9	21.4±1.0*
Peak HR ($b \cdot min^{-1}$)	143±3	141±5	144±4	145±3
V_E peak ($L \cdot min^{-1}$)	51.7±1.8	50.8±2.4	55.2±2.4*	54.2±2.5
VO_2T_{ge} ($L \cdot min^{-1}$)	1.04±0.03	1.01±0.04	1.04±0.04	1.12±0.05*
Peak RER	1.10±0.02	1.11±0.03	1.13±0.03	1.07±0.02

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HbA_{1c}, glycated haemoglobin; TE, time to exhaustion; VO_2peak, peak oxygen uptake; HR, heart rate; V_E, ventilation; VO_2 T_{ge}, oxygen uptake at gas-exchange threshold; RER, respiratory exchange ratio.
*, $p < 0.05$, significantly different to pre-training (wk 0).

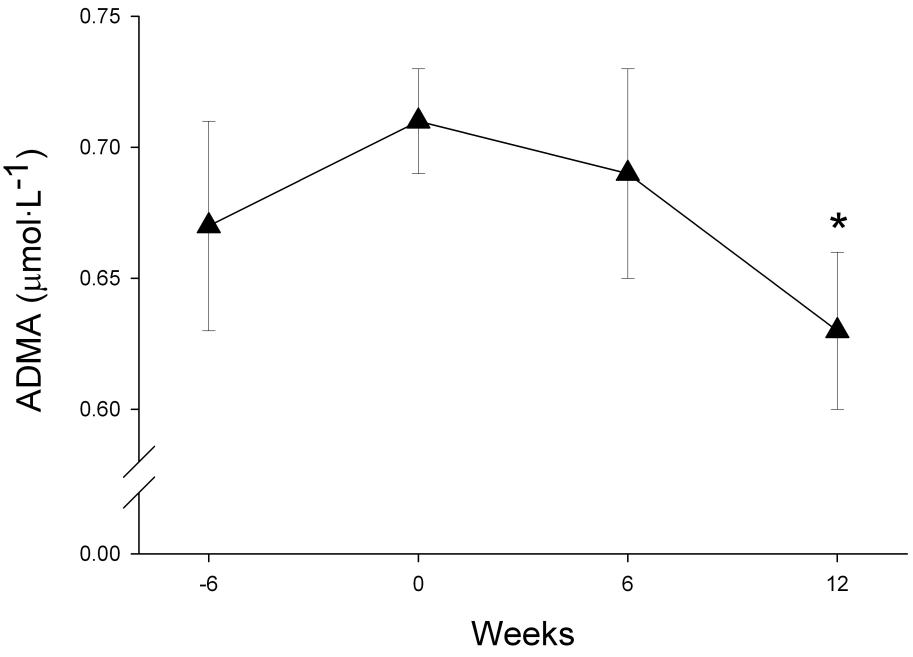


Figure 1. Changes in plasma ADMA concentration (▲) during 6-week control period (wk -6 to wk 0), and after 6 and 12-weeks of exercise training (wk 0 to wk 6 to wk 12). Mean ± SEM. *, $p < 0.05$, significantly different to pre-training (wk 0).

CHAPTER FIVE

General Discussion and Conclusions

Although the risk of disease and disability clearly increases with advancing age, poor health is not an inevitable consequence of aging.....Proven, effective strategies to prevent chronic disease, disability, and death exist, but they have not been widely used. Physical activity is the key to healthy aging. Nowhere is the gap wider between what we know and what we do than in the area of physical activity. Nowhere is the potential payoff greater.

- *(Koplan 2000)*

CVD is a major cause of morbidity and mortality in older individuals with type 2 diabetes (Astrup 2011). While the benefits of regular exercise on vascular function in individuals with type 2 diabetes are well established (Maiorana et al. 2001; Okada et al. 2010), the mechanisms behind these benefits are unclear. Thus, the primary aim of this thesis was to understand select responses in vascular structure (RVC and D_f) and ADMA concentration to moderate-intensity exercise (walking) in women aged 65-74 yr with type 2 diabetes. Data from this study may help in establishing measurements of retinal microvascular structure (RVC and D_f) and AMDA concentration as novel therapeutic targets for the prevention of CVD in older patients with type 2 diabetes.

5.1 Review of Findings

In Chapter Two (Study One), we report the relationship between measures of physiological functional capacity and retinal microvascular structure in older women with and without type 2 diabetes. Until the present study, the relationship between VO_2peak and TE, direct measures of PFC and retinal microvascular structure had not been examined. Thus, it is unknown whether moderate-intensity exercise has long-term beneficial effects on the retinal vasculature. In the present study no significant relationship was found between any measures of PFC and retinal vessel calibre. However, a significantly higher D_f was found to be positively correlated with time to exhaustion in subjects with type 2 diabetes. These results are not surprising given that quantifying retinal vascular complexity using D_f has been suggested to provide a more accurate and sensitive overall estimate of retinal microvascular structure than vessel

calibre alone (Liew et al. 2008b). The results of our study suggest that retinal vascular branching pattern is more complex in those individuals who had a longer time to exhaustion during graded exercise testing. Further investigations involving a larger, more diverse population with regards to PFC are required to determine if any other relationships between retinal microvascular structure and measures of PFC exist.

Chapter Three (Study Two) expands on the results of Chapter Two (Study One) by investigating the effects of a 12-week exercise intervention (walking) on RVC and D_f in women aged 65-74 years with type 2 diabetes. While a few studies have examined the relationship between physical activity and retinal microvascular structure (Anuradha et al. 2011a; Anuradha et al. 2011b; Anuradha et al. 2011c; Tikellis et al. 2010), it was previously unknown whether supervised, moderate-intensity exercise would have long-term beneficial effects on the retinal vasculature. In our study, 12-weeks of supervised, moderate-intensity walking at an intensity equivalent to T_{ge} did not result in significant changes in mean RVC or D_f, despite significant increases in VO_2peak, TE, VO_2 at T_{ge}, as well as significant reductions in systolic and diastolic blood pressure. These results suggest that retinal microvascular structure may not be a useful clinical marker to measure the effectiveness of exercise as a therapeutic intervention for the prevention of CVD in older women with type 2 diabetes, or that the duration of the exercise training program needs to be expanded. Further investigation with a longer duration of exercise and a range of exercise intensities may help to clarify the relationships between retinal microvascular structure and physical activity.

In Chapter Four (Study Three), the effects of moderate-intensity exercise on another potential clinical marker of CVD risk, ADMA, was examined in the same cohort as the previous two studies. ADMA is elevated in patients with type 2 diabetes and thought to play a role in the endothelial dysfunction and increased CVD risk associated with type 2 diabetes (de Jager et al. 2006; Malecki et al. 2007; Yamagishi et al. 2008). While exercise has been shown to reduce ADMA concentration in certain populations (Gomes et al. 2008; Mittermayer et al. 2005; Richter et al. 2005), no studies to date have examined the effects of exercise on ADMA concentration in individuals with type 2 diabetes. In our study, plasma ADMA concentration was found to be significantly lower after 12-weeks

of moderate exercise training, suggesting that ADMA may play a role in the training-induced reduction in cardiovascular disease risk seen with exercise training in individuals with type 2 diabetes. Importantly, these reductions in ADMA were not accompanied by significant improvements in homocysteine concentration and may therefore act as a more sensitive marker of CVD risk in medically well-managed patients who may not show more conventional signs of CVD risk.

5.2 Discussion

Currently, the American Diabetes Association suggests that individuals with type 2 diabetes should follow physical activity guidelines outlined for the general population (American Diabetes Association 2012b). The American Diabetes Association recommends that individuals with diabetes, regardless of age, should "perform at least 150 min/week of moderate intensity aerobic physical activity (50-70% of maximal heart rate)". The above guidelines are primarily influenced by studies examining the effects of exercise on glycaemic control in individuals with type 2 diabetes (Boule et al. 2001; Boule et al. 2003). To date, few studies have focused on examining the effectiveness, and optimal dosing strategies of exercise on improving markers associated with the complications of diabetes. Further studies are required to establish a minimal therapeutic dose of exercise for protective cardiovascular responses in older, female individuals with type 2 diabetes. Based on our difficulty recruiting subjects to volunteer for this particular exercise training study, many older women may not be willing to commit to 150 min/week of moderate intensity aerobic physical activity. After a significant recruitment campaign over a 24-month period starting in 2007, only 20 women meeting inclusion criteria volunteered to complete the testing procedures and exercise training program. After very little response from newspaper advertisements, radio and television announcements, information booths at local shopping centres, and flyers posted at local pharmacies, medical centres, diabetes education clinics, pathology laboratories, and ophthalmology clinics, a different recruitment strategy was implemented. With the help of Diabetes Australia Queensland, 600 recruitment flyers were sent out to individuals who lived within a short distance to our testing laboratory and who directly matched our inclusion criteria for our study. Despite extensive recruitment efforts, only 55 eligible

individuals expressed interest and attended an information session at Bond University. Of these individuals, only 20 were willing to participate, with most non-participants citing the significant time commitment associated with the study as the reason for not participating. The unwillingness of the vast majority of women in this age group to commit to an exercise training program consisting of 120 min/week suggests that a more convenient and manageable exercise prescription may need to be established for this population.

Additional research, with larger subject cohorts, is required to develop a true dose-response for the beneficial effects of exercise on older women with type 2 diabetes.

Study One and Two are the first studies to examine the effects of increased measures of PFC and a 12-week exercise intervention on the microcirculation in individuals with type 2 diabetes. While 120 min/week of regular exercise at individual T_{ge} did not result in significant changes to the retinal microvasculature, measures of vascular complexity (D_f) were found to be influenced by measures of PFC (TE) in diabetic individuals. Individuals who had a longer time to exhaustion during graded exercise testing to volitional fatigue had more complex microvascular branching patterns. The mechanisms responsible for the relationship between D_f and measures of "cardiovascular fitness" are not clear. The beneficial vascular adaptations to regular exercise have been shown to be largely mediated by changes in vascular structure (Prior et al. 2004), specifically in improvements in endothelial function (Vassalle et al. 2003). Therefore, exercise-induced changes in endothelial function may contribute to the associations between D_f and measures of PFC in our study. It has been suggested that regular exercise training may improve endothelial function by enhancing NO bioavailability (Goto et al. 2003; Green et al. 2004; Maeda et al. 2001). Retinal microvascular tone is mainly regulated by NO and it has been suggested that NO bioavailability may influence retinal arterial vessel structure (Hanssen et al. 2011). While the underlying mechanisms behind the relationship between retinal microvascular structure and NO are likely complex, results from Study Three suggest that systemic ADMA concentrations may play a role in the association between retinal microvascular structure and NO.

Study Three found that in older women with type 2 diabetes, regular, moderate-intensity exercise decreases circulating plasma ADMA concentration. The decrease in systemic ADMA concentration with exercise may have influenced retinal angiogenesis and contributed to the association between D_f and TE observed in Study One. Previous studies have linked retinal fractal dimension to retinal angiogenesis (Masters 2004; Misson et al. 1992). One possible mechanism for the relationship between retinal D_f and angiogenesis is the dysregulation of the ADMA/DDAH pathway, which has been found to be associated with impaired angiogenesis (Fiedler et al. 2009). Further studies are warranted to develop our understanding of the relationship between retinal microvascular complexity and ADMA.

5.3 Conclusions

The findings presented in this thesis support the use of regular, moderate-intensity exercise as an effective intervention for the management of type 2 diabetes in older women. Moreover, the data suggest that:

1 In older women with type 2 diabetes, retinal vascular branching pattern is more complex in individuals with higher "cardiovascular fitness", as measured by time to exhaustion during incremental exercise testing.

2 In older women with type 2 diabetes, twelve weeks of supervised exercise training did not improve retinal microvascular calibre or fractal dimension. These results suggest that changes in retinal microvascular structure may not be a useful clinical tool for measuring the effectiveness of regular exercise as a therapeutic intervention for the prevention of cardiovascular disease.

3 In older women with type 2 diabetes, twelve weeks of supervised exercise training at T_{ge} resulted in significant increases in TE, VO_2peak, VO_2peak relative to body mass, RER, VO_2 at T_{ge}, heart rate at T_{ge} and significant decreases in systolic and diastolic blood pressure.

4 Regular exercise has the potential to decrease systemic ADMA concentrations in older women, which may be a potential mechanism underlying the relationship found between retinal vascular complexity and measures of physiological functional capacity.

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APPENDIX A

Sample Information Sheet and Consent Form

Type 2 Diabetes Exercise Research Project

Gene expression and vascular function in women with Type 2 Diabetes
following 12 weeks of moderate-intensity exercise training

INFORMATION SHEET

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Where will the study be conducted?

The project will be conducted in the Faculty of Health Sciences and Medicine at Bond University. In addition, there may be times when we ask you to visit Griffith University, the Gold Coast Eye Clinic and one of the Gold Coast Pathology Collection Centres.

Why is this research being conducted?

The purpose of this study is to examine the way that Type 2 diabetes affects skeletal muscle and blood vessels. Additionally, we intend to determine how much exercise is required to gain health benefits in Type 2 diabetic women aged 65-74 years.

Why is this important?

It is important to understand how Type 2 diabetes affects skeletal muscle because diabetes has become Australia's fastest-growing chronic disease, with approximately 275 Australian adults being diagnosed every day. We are interested in helping older women because this portion of the population is rapidly growing: in the next decade or so, the number of women aged 65-74 years will almost double. This poses a challenge to our health care system because approximately 75% of older females are either physically inactive or do not exercise frequently.

Regular exercise is known to prevent and manage Type 2 diabetes, however how this happens is still poorly understood. Additionally, if we are to achieve the health benefits associated with regular exercise, we need to know the correct prescription of exercise that should be given to women aged 65-74 years to achieve those health benefits.

This study will investigate the best exercise prescription for Type 2 diabetic women aged 65-74 years

Who will use the information gathered from this study?

The information that we get from your participation in the study, and from the participation of other similarly aged women, will be used by General Practitioners (GP's) and other health professionals such as Physiotherapists and Exercise Physiologists when prescribing exercise as a preventive health measure for their patients.

Do I get a copy of my results and feedback on participation?

Throughout this study, we will be making repeated measurements of your blood, your fitness levels, your heart and muscle function, and your body fat. At regular intervals, we will give you a summary of your results with an opportunity for you to ask questions. If you wish, and with your consent, we can send a summary of your results to your General Practitioner (GP). At the end of your participation, we will give you a summary selection of your results.

Your relationship with your GP

Should you be invited to participate in this study, it is very important that your GP is informed about your participation. Informing your GP about your participation in this study can be done by a letter from us to your GP. Should your GP not support your participation in the study then regretfully, you will not be able to participate.

Before you start

We will invite you to visit Bond University for an information session that will provide you with an in-depth look at what this study will involve. During this visit, you will be familiarised with the procedures being used in the project. This will include the description of various tests that will assess muscle, heart, vascular (e.g. your veins and arteries) and visual function, blood parameters, and body fat. If you wish to continue in the study, we will invite you to sign an informed consent form.

Your health and well-being are of utmost importance to us. Before you embark upon any new physical activity, you should obtain medical advice to ensure that your body can cope with exercise. To ensure your well-being, we will provide you free-of-charge a detailed medical examination that will include a blood test, a physical examination, an examination of your heart and lungs, and measurement of your blood pressure. To ensure your safety, we have implemented very strict medical criteria that you must meet before we can allow you to participate in this study.

If in the opinion of the medical doctor you do not meet our criteria, you will not be able to participate in this study. We take this very seriously to ensure that your health and safety are not at risk. If you are not allowed to participate in this study, we will suggest that you visit your general practitioner to discuss the reasons why you cannot participate. We will provide you with a copy of the medical examination to show your general practitioner.

If you are informed that you have met the medical criteria to continue with this study, we will randomly allocate you to a particular exercise group. We randomly allocate people into groups to ensure that our results are in no way biased. One consequence of this random allocation is that you may be allocated to a group that does no exercise. In our case, the group that does no exercise is called a *control group* and is a very, very important group to this study. In all, there will be 2 exercise groups and 1 control group.

Duration of involvement

If you agree to participate in this project, your time commitment to this study will be substantial. If you are balloted to the exercise training group, your total involvement will be approximately 12 weeks. If you are balloted to the control group, your time commitment will be less. This study involves the collaboration between two of the Gold Coasts' major universities- Griffith and Bond universities. As such, the training study will be conducted in the Faculty of Health Sciences and

Medicine at Bond University. In addition, there may be times when we ask you to visit the School of Physiotherapy and Exercise Science at the Gold Coast campus of Griffith University.

Prior to commencement of the exercise program, you will be allocated to one of three groups by drawing a number from a plastic container. This allocation will determine how many times per week you are required to visit Bond University to exercise and how many minutes of exercise training you will perform each time you visit Bond University.

The table below (Table 1) sets out the number of exercise training sessions per week at Bond University, the number of minutes of walking time per session and the total numbers of weeks of exercise for the three groups.

Table 1. Your time commitment to this project

Group number	Walking sessions per week	Walking time per session	Weeks you will be walking
Group 1	Two	60 minutes	12 weeks
Group 2	Four	30 minutes	12 weeks
Group 3 (control)	No exercise	None	None

If you are allocated to **Group 3**, our control group, you will not be coming to the laboratory each week to walk, but you will still participate in our tests on your blood, fitness level, your heart, muscle, vascular and visual function, and your body fat. If you are a member of the control group, you will be required to visit the laboratory on at least 4 occasions to have these measurements made. In addition, you also may be asked to visit the School of Physiotherapy and Exercise Science at the Gold Coast campus of Griffith University.

If you are allocated to an exercise-training group, you will be required to walk on a motor driven treadmill a number of times per week. You will be asked to attend Bond University and walk for a total of 120 minutes per week on either 2 or 4 times per weeks. During the exercise-training program, you will be required to walk at about 50% of your maximum capacity. This will feel like a brisk walking pace where it is a little difficult to talk freely while exercising.

There will be a substantial commitment of your time to this project

During each exercise training session, your heartbeat will be monitored and recorded. Each exercise training session will include a warm up period at the start (walking, usually for 2

minutes) and a warm down period at the end (walking, for usually 2 – 5 minutes). During the exercise-training program, you will be directly supervised by experienced investigators.

The walking speed and treadmill slope during the exercise-training program will be determined from the peak exercise test (max test). You may find that the speed or slope of the treadmill during the exercise-training program increases throughout the 12 weeks. This is a positive sign that your body is adapting to our exercise-training program and is a healthy positive consequence of your repeated exercise.

What you will be asked to do

Once we have confirmed that it is safe for you to participate in this study, you will be asked to visit Bond University for the first of 3 tests. These 3 tests will be performed 1) before you start-, 2) half way through- (week 6), and 3) immediately after (week 12) the exercise program. These tests will involve an assessment of your fitness; body fat percentage; eye, vasculature, muscle and heart function; and some key blood parameters (how we assess these things will be described in detail below).

These tests will be additional to the exercise training that you will perform. The exercise training is what will help you become more fit and healthy, whereas the tests that we will perform allow us as scientists to measure how much of a difference the exercise is making to your health.

Blood measurements

We will ask you to give us a blood sample on 6 different occasions. The blood will be taken from a vein in your arm by an accredited person that works with an accredited pathology laboratory, similar to the person who your general practitioner uses. The amount of blood taken from the vein in your arm will be approximately 20 milliliters (a normal tea-cup has about 250 milliliters). This is a routine amount of blood that a pathology laboratory would take when your general practitioner orders a blood test for you. During the blood measurement, you may experience some discomfort during the insertion of the needle into your forearm. We will try to minimise the amount of discomfort by employing an experienced and accredited person to take these blood measurements.

Your blood will then be measured for key parameters such as cholesterol, glucose, triglycerides, iron, glycosylated haemoglobin and fructosamine. A full blood count will also be performed. Some blood will also be analysed at Griffith University for markers that let us know the health of your veins.

Eye test

The eye test will be similar to those that you may have experienced while having a routine eye inspection. A series of 4 tests will be performed during a 30 minute period by an experienced and accredited optometrist. This visit will involve 1) measurement of visual acuity (i.e. the ability to see a vision chart); 2) examination of the pupils and eye movements; 3) dilating your pupils with light to examine the lens and retina. Photographs will be taken of the inside of your eye to help us evaluate the status of the retina and evaluate possible changes that may occur due to exercise training. Depending on the condition of your eye, between 2 and 20 photographs may be taken. To obtain the photograph, a bright light is flashed into the eye for each picture. This procedure does not cause discomfort and has not been reported to damage the eye in any way. All tests will take between 45 – 60 minutes to complete at no cost to you. Dr John Kearney of the Gold Coast Eye Clinic will perform these tests. To summarise, these tests will allow us to determine whether regular exercise can help reduce the optical damage caused by Type 2 diabetes.

Assessment of your fitness

We will ask you to walk on a treadmill that will increase in speed and slope until you can do no more, or you tell us you have had enough or if the supervising medical doctor tells us you have done enough. We will call this a peak exercise test- some people call them a 'max test'.

During your peak exercise test, we will monitor your breathing and heart while making some calculations. A medical doctor trained in monitoring people while exercising will be directly monitoring you at all times while performing this test. This test will tell us about your fitness level and how your heart and lungs are functioning during exercise. It will also let us know how much you have improved since commencing the exercise program. This test carries a small risk to your health, so it is important you read the **Risks** section carefully. The procedures for this test are described below.

The "peak exercise test" or "max test" is used to measure your fitness level and your heart and lung function. The "peak exercise test" or "max test" will involve you walking on a treadmill with the speed and slope increasing until you say you wish to stop or the Doctor indicates that you have done enough. Throughout the exercise test, you will have a mouthpiece (a bit like the ones used in snorkels) in your mouth. The mouthpiece will be attached to a tube and we will collect all the air you blow out. The air that you blow out enables us to determine your level of fitness. To maximize the amount of air blown out through the mouthpiece we will place a nose clip over your nostrils to make sure that no air is lost through your nose. The "peak exercise test/max

test" will begin with a warm-up where we can check that all our instruments are working and that your body is responding in the correct and safe manner. The warm-up will be at a walking pace approximately 2.0 km/hr for 3–5 minutes. After 3-5 minutes of warm-up, the walking speed and grade of the treadmill will increase in an alternate manner each minute. As the walking speed and grade increase, you will find that your breathing increases and your heart will beat faster and harder. These are normal responses. As the "peak exercise test/max test" continues, you will find that your legs become heavier, your heart beats faster, and breathing increases; these are all normal responses. There will come a point when you do not wish to continue **walking** on the treadmill. When you decide that you have **walked** enough, you can signal that you have had enough by prearranged hand signals. We will slow the **walking** speed to about the same pace as your warm-up on the treadmill. This slow walking pace after your "peak exercise test/max test" is called the warm down. During the warm down the mouth piece and valve will be removed, and the headpiece supporting the mouthpiece and valve will be removed. A drink of water will be offered after your "peak exercise test/max test".

Measurement of your heart function

The peak exercise test will give us, and you, information about your fitness and your heart and lung function during exercise. During the peak exercise test at about 90% of your capacity, we may ask you to breathe a harmless mixture of oxygen and special gases. By breathing this harmless mixture of oxygen and special gases, we can calculate how much blood your heart pumps out each minute during the peak exercise test. On a separate occasion, we will also ask you to repeat the same heart and lung function test while walking at about 50% of your maximum capacity, and at a fixed walking pace of 2 km/hr while walking up a small slope (i.e. up-hill at ~1% grade).

Measurement of your body fat percentage

Your body fat will be estimated by taking some measurements of the amount of fat in the front and back of your arm, on the top of your hips and at the bottom of your shoulder blade using calipers specially designed for this purpose. You may feel a very slight discomfort while the calipers are measuring the amount of fat under your skin. After a few seconds, the pressure in the calipers is released and all discomfort will disappear. These measurements are referred to as skinfold measurements, or "the pinch test", and will be performed by a female investigator trained in taking skinfold measurements. The circumference of your waist and hips will be measured by a female research assistant, using a flexible (not steel) measuring tape.

Measurement of muscle proteins and genes

On a separate occasion, we may ask you to visit the laboratory for us to obtain a small (100 milligram) sample of your leg muscle, using a sterile hollow needle. This procedure is called a muscle biopsy. Muscle biopsies are a commonly performed procedure in research projects that allow us to investigate much more thoroughly how diabetes affects you. Considering that type 2 diabetes is considered a metabolic disease, the measurement of muscle proteins and genes will allow us to investigate how and why exercise improves insulin sensitivity and blood glucose in diabetics. This information may aid us in finding the optimal amount of exercise required to manage diabetes and also aid the search for a cure.

Before a biopsy is taken, the risk and benefits will be explained to you by our team. We must stress that this is completely voluntary and you may refuse to continue with the biopsy at any time, with no consequence to your involvement in the study. The procedure for obtaining muscle from your leg will involve an experienced medical doctor injecting a small amount of local anaesthetic into the skin of your thigh after your leg has been carefully cleaned. The doctor will then make a small (4 - 5mm) incision in your skin to create an opening for the biopsy needle. There is often a small amount of bleeding from this incision, but it is usually minimal.

The biopsy needle will then be inserted through the incision into the thigh muscle. The biopsy needle uses suction to take a small portion (100 – 200 mg), about the size of a pea, of muscle from your vastus lateralis- one of your thigh muscles. While the needle is in your thigh (about 5 seconds) you may feel the sensation of deep pressure in your thigh, and on some occasions this is moderately painful. However, the discomfort passes very quickly and you are quite capable of performing daily activities soon after.

The doctor will then surgically dress and bandage the incision site. You will then be monitored for an hour in the laboratory to ensure your well-being. You should refrain from excessive muscle use for the remainder of the day. Once the anaesthetic wears off, you may feel the sensation of a deep bruise in your leg. This is normal and you may use an over-the-counter pain killer (such as ibuprofen) if you experience any pain associated with the biopsy. It is also beneficial to apply an ice pack to the biopsy site the following day to reduce swelling and soreness.

We will call you each day for the following 3 days to ensure that you are doing well. You will also be welcome to attend the laboratory at any time to have the site checked if you have any concerns. Any discomfort you may experience usually disappears within 2 days. Daily showers are acceptable, but baths, swimming, saunas etc. should be avoided for at least 4 days following the procedure.

The muscle sample will be analysed for various proteins and genes that are involved in the development of type 2 diabetes. We want you to know that your safety is our utmost interest. In previous studies, our physician and research team have performed this technique with no complications.

Measurement of vascular function

The thickness of some of your arteries will be measured using an echo-doppler ultrasound-similar to one that you may have seen at a physiotherapist. You will also undergo a limb blood flow test. Please abstain from caffeine and alcohol for 24 hours before your appointment at the study centre.

For the blood vessel measurement, we will be measuring the diameter of the carotid artery (along the neck), brachial artery (along the inner arm) and femoral artery (along the inner thigh, just below the groin). An ultrasound machine will be used to take an image of the arteries while you are lying down in a supine position. There is no pain involved in the ultrasound examination. You may feel a slight pressure as the ultrasound probe is placed and moved along your skin; and the ultrasound gel will feel momentarily cold when it is first applied to your skin. The ultrasound gel is safe and not known to cause skin irritation. The gel is water-soluble and wipes off easily. The measurements should take about 30 minutes to perform.

During the blood flow tests, a pressure cuff (like a blood pressure cuff) will be placed around your thigh and upper arm. The cuff-pressure will be increased and decreased intermittently to measure blood flow through your calf, thigh and forearm muscle. This procedure will be repeated several times. On some trials, the cuff will be inflated to a high pressure, and this pressure maintained for up to 5 minutes. During this time, blood flow to the limb is transiently disrupted. Many people find this procedure uncomfortable and even painful. You may experience a tingling sensation, “pins and needles” and some degree of numbness in your lower limb or arm. However, this sensation will subside very rapidly after the cuff is deflated and there will be no long-term negative health effects from this procedure. The upper limb (arm) and lower limb (thigh and calf) blood flow tests will be performed separately, with at least 10-minute rest periods in between. The entire blood flow test procedure should take between one, and one and a half hours to perform.

The expected benefits of the research.

By participating in this study for 12 weeks, you will get a significant amount of information on your health, and how your body adapts to repeated bouts of exercise. If you complete our 12 week exercise programme, you should expect to see health benefits that include:

- An increase in your fitness;
- A decrease in your body weight;
- A decrease in some of the fats (triglycerides) in your blood;
- A decrease in the stickiness of your blood;
- A decrease in your blood pressure;
- An improvement in your blood sugar level;
- An improvement in your insulin response;
- An improvement in your feeling of well being.

Benefits of your participation in the Control Group

By participating in our study as a member of the Control Group, you will still get a significant amount of information with explanation on your health. As a member of the Control Group, you will be providing a very important comparison point. Indeed, without a strong and committed Control Group, this project is of considerably less value.

Risks to you

With the right preparation, the “peak exercise test’ or “max test” is a very safe procedure. Even with the right preparation, there is a very small risk that a misadventure could occur. The misadventure may include falling over, dizziness, heart attack and death. The American College of Sports Medicine states, “exercise only provokes cardio-vascular (heart) events in individuals with pre-existing heart disease. Exercise does not provoke cardiac (heart) events in individuals with normal cardio-vascular systems”. Before you begin any exercise, including the “peak exercise test”, we will ask you to have a detailed medical examination, including a blood test, a lung function test and a resting electrocardiogram (ECG). All these tests and the detailed medical examination are intended to prepare you for the “peak exercise test” and to determine if you have clinically significant cardio-vascular disease. Our preparation is to ensure your safety and well-being during all our exercise tests. Any exercise test and any exercise-training

program carry some risk to your health and well-being. Our processes and procedures are designed to minimize any risk of heart, lung, muscle and bone misadventure.

Exercise related cardiac events in adults

The risk of misadventure including cardiac events during exercise is higher in older adults than in young adults. The overall risk of exercise testing in a mixed subject population is approximately 4-6 cardiac events per 10,000 exercise tests. Cardiac events include heart attack, very rapid and irregular heartbeats and death.

With the correct pre-screening (preparation) before the “peak exercise test/max test”, and with your heartbeat and heart rhythm closely monitored during the “peak exercise test/max test” by a medical doctor, the risk of cardiac misadventure is extremely small.

In Table 2, (below) is information on the number of cardiac events that have occurred per 10,000 exercise tests.

Table 2. Information on the number of cardiac events during exercise testing

Number of exercise tests	Heart attack	Irregular heart beat	Death	Hospitalization
170,000	None	None	1/10,000 tests	3/10,000 tests
12,000 (hospital)	None	None	None	None
50,000	0.8/10,000 tests	0.8/10,000 tests	6.4/10,000 tests	5.2/10,000 tests
28,133 (Cardiology practice)	1.42/10,000 tests	1.77/10,000 tests	0	0

Risk of cardiac events during exercise training programs (brisk walking)

The risk of cardiac/heart incidents (heart attack, heart arrest, death) in people who participate in a supervised cardiac rehabilitation programme (similar to our exercise training program) is extremely low. In supervised cardiac rehabilitation or exercise training programs (similar to our exercise training program), the incidence of cardiac events is reported to be:

One cardiac arrest per 116,906 persons;

One heart attack per 219,970 persons;

One death per 752,364 persons.

Our strategies to minimize risk of a cardiac event

We will minimize the risk of a cardiac (heart) event during your "peak exercise test/max test" and during your exercise training program by asking you to complete the following:

1. A modified physical activity readiness questionnaire (PAR-Q). The PAR-Q is a medical questionnaire of approximately 8 questions which can be answered by Yes or No responses, and provides preliminary information about your health
2. A medical questionnaire detailing your medical history, your family medical history and your smoking, drinking, and physical activity habits. You will also be asked about the tablets or medication you take including those that you buy over the counter at the Chemist Shop or Health Food Shop, and those that are prescribed by your Doctor and issued by your Chemist
3. A detailed medical examination by a medical doctor
4. A preliminary and moderate walking test on a motor driven treadmill (walking platform) with direct medical supervision and direct monitoring of your heart and breathing. Staff experienced with conducting exercise tests and operating exercise testing equipment will be in attendance with the medical doctor during the preliminary and moderate walking test. The preliminary walking test is part of the detailed medical examination to ensure that the condition of your heart, lungs and muscles will allow you to be allocated to one of our exercise or control groups
5. 5-7 days after the preliminary walking test, we will ask you to visit us again for a "peak exercise test" (max test) on a treadmill with direct medical supervision and direct monitoring of your heart and breathing. Staff experienced with conducting exercise tests and operating exercise-training equipment will assist the medical doctor with the "peak exercise test"
6. The exercise-training program will be supervised by appropriately qualified and certified persons. There will be direct monitoring of your heart rate and heart rhythm during each exercise training session

7. Whenever you are undertaking a “peak exercise test” or an exercise training session (walking programme), there will be a medical emergency cart on site. This medical emergency cart contains medial drugs, oxygen and equipment that would be used should you have a cardiac event or misadventure occur. Support will also be provided by persons who have received accreditation in CPR and First Aid.

Other risks associated with your participation

During the initial stages of your participation in the project, you may get some muscle soreness, particularly if you have not been exercising those muscles associated with walking. The muscle soreness is sometimes called delayed onset muscle soreness and typically appears 12-24 hours after the exercise. Delayed onset muscle soreness is a normal response and should resolve itself within 36-72 hours.

The risk of falling during the “peak exercise test/max test” to measure your fitness, and heart and lung function will be essentially eliminated by the use of an upper body harness suspended from the roof/ceiling. During the exercise training program, the speed of walking is estimated to be a brisk walk. The risk of falling during the exercise training program on a motor driven treadmill will be reduced by instruction on correct technique of walking on a treadmill, getting on and off the treadmill and the use of front and small side rails on the treadmill for support.

The **muscle biopsy** carries some risks due to the nature of the procedure. As is our past practice, an experienced physician will take all muscle biopsies using aseptic techniques with sterilised instruments and biopsy needles. You will be allowed to refuse the biopsy procedure at any stage of the study. Following the biopsy the wound will be surgically dressed and bandaged. You will then be monitored for one hour in our laboratory. While we have had no previous complications with this procedure, we will continue to monitor your progress over the phone each day for the next 3 days. If you have any concerns with the procedure, you may visit the laboratory at any stage to have the site checked.

Some risks associated with muscle biopsy include:

- You may feel a burning feeling in the thigh during the time of inject. This will last only 5 – 10 seconds. There is also an extremely low risk of allergic reaction to the local injection (1 in 1 million)
- There is a chance (less than 1 in 1000) of a local skin infection at the site of the biopsy. By carefully cleaning the skin and keeping the area clean and dry until the skin heals, the chance of infection in minimised

- Most people will experience local soreness in the leg for 2 – 3 days following the biopsy- this feels like a deep bruise. There is also a very low risk of internal bleeding at the biopsy site which can result in prolonged pain and stiffness in the leg
- On occasion, a small lump of scar tissue may form under the site of the incision, but this normally disappears within 2 - 3 months, or within a few weeks if massaged. A small visible scar often remains from the biopsy incision
- There is the possibility of a small area of numbness (about the size of a 20 cent coin) around the biopsy site. This usually resolves over 5 – 6 months. There is a very low risk (estimated at less than 1 in 5000) of damage to a small nerve branch to the muscle. This would result in partial weakness of the vastus lateralis muscle (one of four muscles that straightens the knee) and would likely have no impact on day-to-day activities. Nerve injuries like this usually resolve in 8 – 12 months, but there is a theoretical risk of mild leg weakness.

Your confidentiality.

The information that you provide and the investigators collect during the study will be treated as confidential. Only the investigators named on this form will see your name and results together. Your anonymous results will be added to the anonymous results from other participants and the combined anonymous results of all participants may be reported in scientific journals and at scientific conferences. The anonymous and combined results will also be given to GP's and other health professionals as professional advice.

Your participation is voluntary.

Your participation in this study is based solely on your voluntary consent, which may be withdrawn at any time without penalty. You do not have to provide reasons for your withdrawal.

Questions/further information.

If you require clarification or have questions about any aspect of this study, do not hesitate to contact one of the investigators. The name and contact details of each investigator is provided on the front of this form.

The ethical conduct of this research

This research abides by the National Statement on Ethical Conduct in Research Involving Humans. If you have any concerns with the ethical conduct of the research party, feel free to contact the Griffith University Manager of Research Ethics by phone on (07) 3875 5585 or email research-ethics@griffith.edu.au or the Bond University Research Human Research Ethics Committee by phone on (07) 5595 4194 or email buhrec@bond.edu.au

Feedback to you

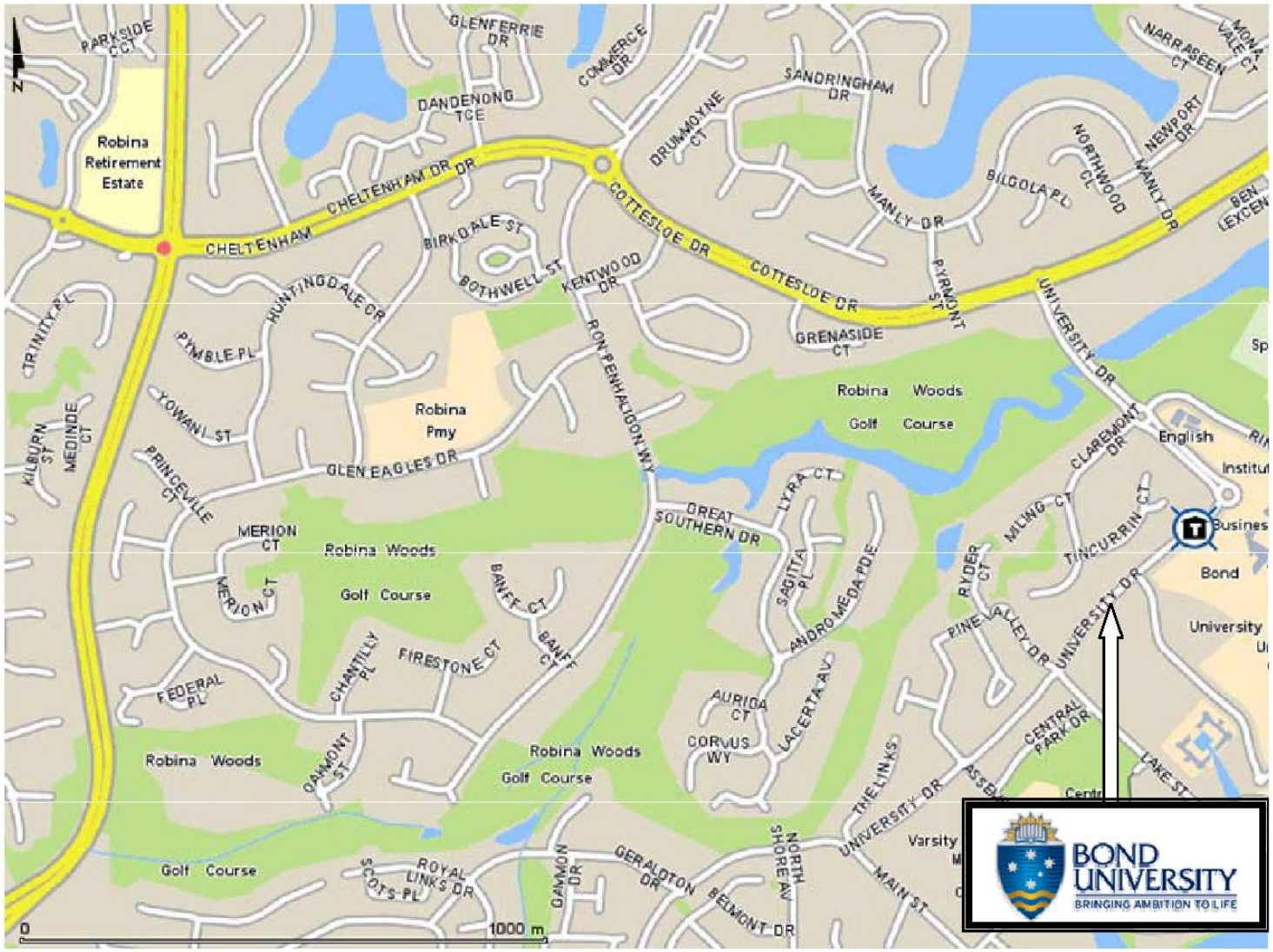
On completion of the study, you will receive a report of your results if requested. In addition, feel free to ask questions at any time before, during or after the study.

Privacy Statement

The conduct of this research involves the collection, access and/or use of your identified personal information. The information collected is confidential and will not be disclosed to third parties without your consent, except to meet government, legal or other regulatory authority requirements. A de-identified copy of this data may be used for other research purposes. However, your anonymity will at all times be safeguarded. For further information consult the University's Privacy Plan at www.gu.edu.au/ua/aa/vc/pp or telephone (07) 3875 5585.

Facilities at Bond University

Change rooms with showers are available close to the area where the exercise-training program will be conducted. Light refreshments, tea, coffee, water and biscuits will be available for you after you finish each exercise training session. There is a comfortable and private waiting area, which is adjacent to the area where the exercise-training program will be conducted. Parking will be made available for easy access to our building. Our building is the Faculty of Health Sciences and Medicine Building and is located on the southern side of the campus. A map of Bond University is attached with directions.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COTTESLOE DR
DANDENONG TCE
GLENFERRIE DR
COMMERCE DR
SANDRINGHAM DR
DRUMMOYNE CT
MANLY DR
BILGOLA PL
NORTHWOOD CL
NEWPORT DR
NARRABEEN CT
MONA VALE CT
BIRKDALE ST
BOTHWELL ST
KENTWOOD DR
HUNTINGDALE CR
PYMBLE PL
TRINITY PL
KILBURN ST
MEDINDE CT
YOWANI ST
PRINCEVILLE CT
GLEN EAGLES DR
RON PENHALIGON WY
GRENASIDE CT
PYRMONT ST
UNIVERSITY DR
Robina Pmy
Robina Woods Golf Course
MERION CT
BANFF CT
FIRESTONE CT
CHANTILLY PL
FEDERAL PL
OAKMONT ST
GREAT SOUTHERN DR
LYRA CT
SAGITTA PL
ANDROMEDA PDE
AURIGA CT
CORVUS WY
LACERTA AV
RYDER CT
MILING CT
CLAREMONT DR
TINCURRIN CT
PINE VALLEY DR
CENTRAL PARK DR
LAKE ST
THE LINKS
ROYAL LINKS DR
SCOTS PL
GAMON DR
GERALDTON DR
BELMONT DR
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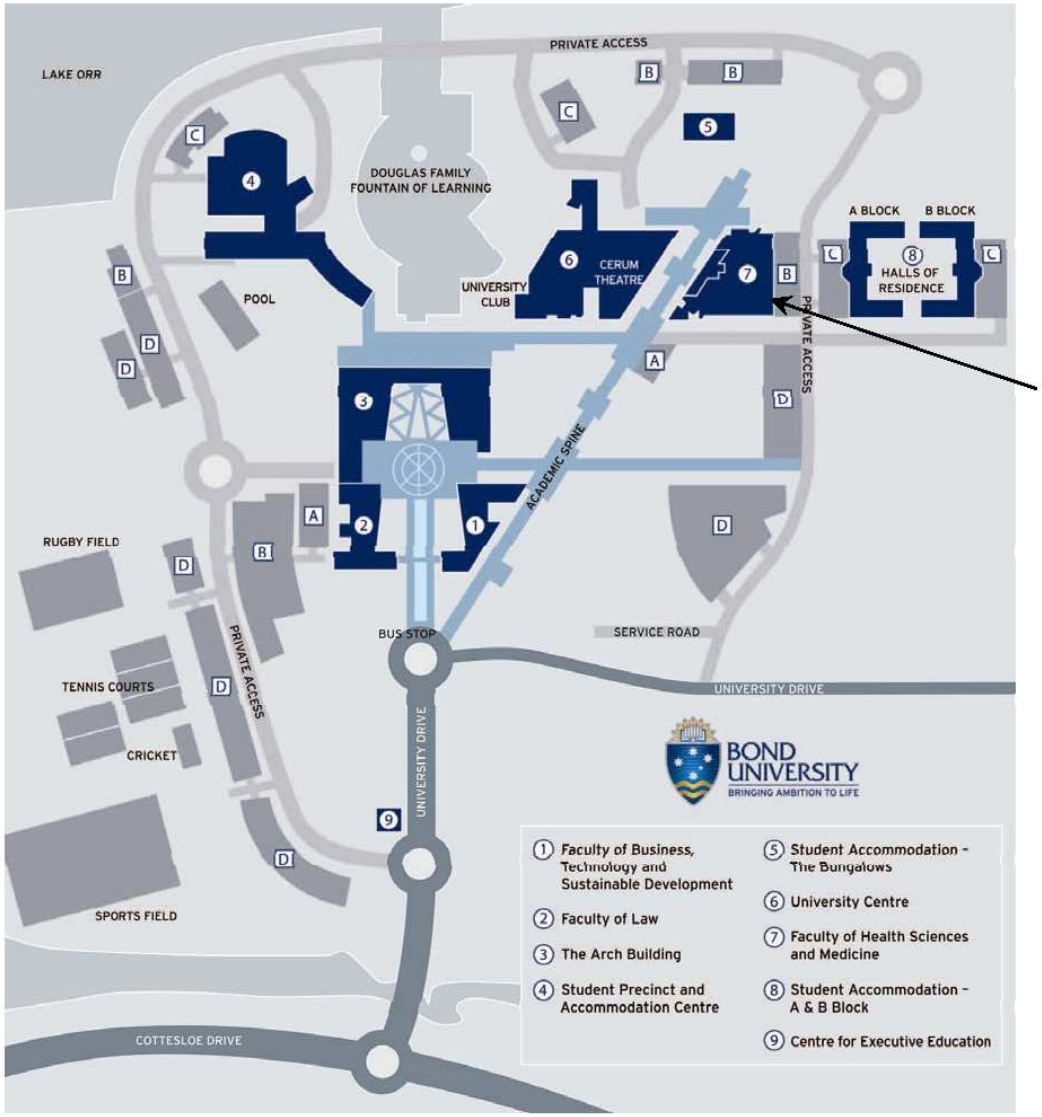

Directions to the testing/training facilities

From Cottesloe Drive, turn into University Drive. Proceed straight along University Drive until you reach the second roundabout (you will see a large arched building in front of you, near a bus-stop). Enter the roundabout and exit to the right (still University Drive).

Take the first left turn (Service Road) once you have exited the roundabout (about 150 metres from roundabout).

Follow Service Road until the Faculty of Health Sciences and Medicine building (building #7 on the map) is to your left (large white building with many dark windows; directly across from ABC child centre).

Parking is not allocated, thus you can park throughout any of the available areas (depicted as 'D' and 'C' on the map).

A member of the research team will greet you at the entrance to the Faculty of Health Sciences and Medicine building and escort you to the meeting room.

Type 2 Diabetes Exercise Research Project

Gene expression and vascular function in women with Type 2 Diabetes following 12 weeks of moderate-intensity exercise training

CONSENT SHEET

Primary Investigators

Prof Greg Gass
Bond University
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Dr Clare Minahan
Griffith University
Telephone: 07 5552 8390

Dr Surendran Sabapathy
Griffith University
Telephone: 07 5552 8281

Dr Luke Haseler
Griffith University

Student Investigators

Michael Simmonds
Griffith University
Telephone: 07 5552 9208

Kevin Serre
Bond University

By signing below, I confirm that I have read and understood the information package and in particular have noted that:

- ☐ I understand that my involvement will include the completion of several exercise sessions each week for twelve weeks, as well as regular testing to assess my health and progress;
- ☐ I have had any questions answered to my satisfaction;
- ☐ I understand the risks involved;
- ☐ I understand there will be no direct benefit to me from my participation in this research;
- ☐ I understand that my participation in this research is voluntary;
- ☐ I understand that if I have any additional questions I can contact the research team;
- ☐ I understand that I am free to withdraw at any time, without comment or penalty;
- ☐ I understand that I can contact the Griffith University Human Research Ethics Committee on (07) 3875 5585 (or research-ethics@griffith.edu.au) or the Bond University Human Research Ethics Committee on 07 55954194 (or buhrec@bond.edu.au) if I have any concerns about the ethical conduct of the project;
- ☐ I understand this project will meet the National Statement on Ethical Conduct in Human Research (Privacy), at http://www.nhmrc.gov.au/publications/synopses/e72syn.htm; and,
- ☐ I agree to participate in the project.

_____	_____	_____
Participant	Signature	Date
_____	_____	_____
Witness	Signature	Date

APPENDIX B

Medical History Questionnaire

Type 2 Diabetes Exercise Research Project

MEDICAL HISTORY QUESTIONNAIRE

Name: _____

Address: _____

_____

Phone: ( ) _____ (W)

Phone: ( ) _____ (H)

Age: _____

DOB: _____

1. **Family history.** Indicate if any of your immediate family (grandparents, parents, brothers, sisters) have experienced any of the following: list their age and relationship to you.

Relationship & age

Heart Disease _____

Stroke _____

Diabetes _____

Cancer _____

2. **Personal medical history.** Indicate symptoms that apply to you.

☐ Pain or discomfort in chest following exercise, eating or exposure to cold
☐ Frequent heart palpitations or flutter
☐ Very poor exercise tolerance
☐ Frequent dizziness

3. Are you presently experiencing, or have you ever been treated by a doctor for any of the following? **Allergies, Hay fever, eczema, other rashes.**

☐ Yes
☐ No
Details _____

4. **Lung problems** (Asthma/Emphysema/Bronchitis/Shortness of Breath/Other)

☐ Yes
☐ No
Details _____

5. **Heart problems** (Rheumatic fever/Chest pains/Palpitations/Ankle swelling/Other)

☐ Yes
☐ No
Details _____

6. **Blood pressure problems**

☐ Yes
☐ No
Details _____

7. **Cholesterol problems**

☐ Yes
☐ No
Details _____

8. **Gut problems** (Ulcer/Abdominal pain/Diarrhoea/Constipation/Hernia/Other)

☐ Yes
☐ No
Details _____

9. **Unexplained weight loss**

☐ Yes
☐ No
Details _____

10. **Urinary problems** (Burning/Difficulty with control of urine)

☐ Yes
☐ No
Details _____

11. **Blood loss** (In Vomit/Sputum/Bowel action/Urine)

☐ Yes
☐ No
Details _____

12. **Easy bruising**

☐ Yes
☐ No
Details _____

13. **Endocrine problems** (Diabetes/Thyroid/Other)
☐ Yes
☐ No
Details _____

14. **Fitting, fainting, blackouts, loss of consciousness, muscle weakness, loss of sensation, epilepsy**
☐ Yes
☐ No
Details _____

15. **Headaches**
☐ Yes
☐ No
Details _____

16. **Sight or hearing problems**
☐ Yes
☐ No
Details _____

17. **Nervous conditions**
☐ Yes
☐ No
Details _____

18. **Bone or joint injury** (Back/Knee/Ankle/Hip/Shoulders)
☐ Yes
☐ No
Details _____

19. **Other joint problems** (aches or pains/arthritis)
☐ Yes
☐ No
Details _____

20. **Work related injuries**
☐ Yes
☐ No
Details _____

21. **Sleeping patterns.** How many hours do you sleep on average per night?
_____ hours

22. **Do you ever have trouble falling asleep?**
☐ Yes
☐ No
☐ Occasionally

23. **Smoking status**
☐ Never smoked
☐ Quit smoking more than ten years
☐ Quit smoking less than ten years
☐ Currently smoke (number of years _____)

24. **If currently smoking**, how many cigarettes do you currently smoke per day?
_____

25. **Physical activity.** How many times per week do you perform exercise such as walking/running, cycling, swimming or organised sporting activities for at least 20-30 minutes continuously?
☐ Do not have a regular program
☐ Once per week
☐ 2-3 times per week
☐ 4-5 times per week
☐ more than 5 times per week

26. On average, how would you **rate the intensity of the exercise that you perform**?
☐ *Light* – Slight or minimal increase in perceived effort and breathing intensity (able to comfortably hold a conversation while exercising)
☐ *Moderate* – Noticeable increase in perceived effort and breathing intensity (but still able to hold a conversation while exercising)
☐ *Heavy* – High level of effort and heavy breathing (unable to comfortably hold a conversation while exercising)

27. **Alcohol consumption.** In the past two weeks list how many days you consumed an alcoholic beverage.
☐ Did not drink in the past 6 months
☐ Did not drink in the past 2 weeks
☐ 1-2 days
☐ 3-4 days
☐ 5-7 days
☐ 8-10 days
☐ 11-14 days

28. In the past two weeks **list how many alcoholic beverages** on average you had per day.
☐ Did not drink in the past 6 months
☐ Did not drink in the past 2 weeks
☐ 1 drink
☐ 2-3 drinks
☐ 4-6 drinks
☐ 7 or more drinks

Medications. Please list all medications you take on the table below.

Name of Drug	**What is it for?**	**Dose**	**Frequency**	**Started Year/date**
e.g. Accupril	*Hypertension*	*20mg*	*Once a day*	*April 2003*
Prescription Drugs				
Over-the-counter medication				
Vitamins and other medications (especially Calcium)				
All medications discontinued in past 6 months				

Operations. Please list all surgeries you have undergone including the approximate date or year.

Surgery	**Diagnosis**	**Date/year**

MEDICAL HISTORY QUESTIONNAIRE

Emergency Contact Form

Name: _____

In case of emergency I authorise the research team, or a representative thereof, to contact the following persons.

Person One:

Name: _____

Relationship: _____

Phone Number(s): _____

Person Two:

Name: _____

Relationship: _____

Phone Number(s): _____

Official Use - Notes

MEDICAL HISTORY QUESTIONNAIRE

APPENDIX C

Physical Activity Readiness Questionnaire

Type 2 Diabetes Exercise Research Project

Physical Activity Readiness Questionnaire (PAR-Q)

For most people, physical activity should not pose any problem or hazard. The PAR-Q has been designed to identify the small number of adults for whom physical activity might be inappropriate or those who should have medical advice concerning the type of activity most suitable.

Read the questions carefully and answer each one honestly, checking **YES** or **NO**.

YES	NO		
☐	☐	1.	Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor?
☐	☐	2.	Do you feel pain in your chest when you do physical activity?
☐	☐	3.	In the past month, have you had chest pain when you were not doing physical activity?
☐	☐	4.	Do you lose your balance because of dizziness or do you ever lose consciousness?
☐	☐	5.	Do you have a bone or joint problem that could be made worse by a change in your physical activity?
☐	☐	6.	Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?
☐	☐	7.	Do you know of any other reason why you should not do physical activity?

I have read, understood and completed this questionnaire. Any questions I had were answered to my full satisfaction.

Name _____ Date _____

Signature _____

APPENDIX D

Example of GP Letter

Type 2 Diabetes Exercise Research Program

Date

Dear **Subject Name**,

Thank you for your continued interest and enthusiasm in our Type 2 diabetes exercise research program. We would now like to invite you to commence the first Pre-training Testing Session. However, before commencing your participation in our project, we ask that you obtain medical clearance from your own doctor (i.e., your GP) to participate in the program.

We have included with this letter: i. a copy of your blood results, ii. a copy of all your pre-exercise screening information, iii. a letter for your GP to sign indicating his/her recommendation about your participation in the exercise program, and iv. a reply postage-paid envelope for your GP to return their recommendation to us.

Please visit your GP within the next 5-7 days with all pre-exercise screening information in the envelope for his/her inspection. Your GP then will be able to make the recommendation about your participation in our research program.

We appreciate your continued interest for our project. If you have any questions regarding this letter, please do not hesitate to contact Kevin Serre on 5595 4048.

Yours sincerely,

Prof Greg Gass
Chief Investigator
Bond University
Telephone: (07) 5595 4481
Email: ggass@bond.edu.au

Dr Clare Minahan
Chief Investigator
Griffith University
Telephone: (07) 5552 8531
Email: c.minahan@griffith.edu.au

Date

Re: **Subject Name**
«Address»
«Suburb» «Postcode»

Dear Doctor,

Request for your recommendation regarding the suitability of *Subject Name*'s participation in our research project involving an incremental exercise test to volitional fatigue and a 12-week exercise training (walking) program.

Subject Name has volunteered to participate in our research project. Our project is running in parallel to a project funded and supported by the Department of Health and Ageing, and will be conducted by Bond University and Griffith University. The program is about determining the optimum exercise prescription for Type 2 diabetic women aged 65-74 years to improve their health and well-being.

To determine the optimum exercise prescription for health benefits in Type 2 diabetic women aged 65-74 years, participants will take part in a 12-week walking program. Before, during, and at the end of the 12-week walking program, we will ask participants to undergo select medical tests, blood tests and exercise tests. Our 12-week walking program will involve walking on a treadmill from 1 to 5 days a week. We will be asking participants to exercise for a total of 120 minutes every week for 12-weeks at an intensity that is equivalent to a brisk walk.

By participating in our research project, **Subject Name** will be asked to undergo an incremental exercise test to volitional fatigue (i.e., Stress Test) under direct medical supervision with immediate access to a fully equipped emergency/resuscitation cart. We are aware that exercise stress testing of this nature does carry some risk of cardiovascular and/or musculoskeletal misadventure. We are requesting your recommendation regarding **Subject Name**'s suitability to participate in our project. We have included a copy of the pre-exercise screening information collected from **Subject Name** to date.

This Pre-exercise screening information includes:

- A medical history questionnaire,
- Selected blood analyses,
- Resting 12-lead ECG data, and
- Resting spirometry results.

In making your recommendation, the research team asks that you carefully consider **Subject Name**'s medical history, and in particular:

a. «Problem_1»
b. «Problem_2»
c. «Problem_3»
d. «Problem_4»

We have also included the exercise program's information sheet that details the walking program, scheduled tests, and participant responsibilities. If you have any further questions you would like answered, please contact Kevin Serre on 5595 4048.

We would appreciate it if you could complete the attached GP recommendation form and return it in the reply-paid envelope provided, at your earliest convenience.

Yours sincerely,

Prof Greg Gass
Chief Investigator
Bond University
Telephone: (07) 5595 4481
Email: ggass@bond.edu.au

Dr Clare Minahan
Chief Investigator
Griffith University
Telephone: (07) 5552 8531
Email: c.minahan@griffith.edu.au

Type 2 Diabetes Exercise Research Program - GP RECOMMENDATION

The NH&MRC project exclusion criteria is provided below.

Cardiac

- A recent significant change in resting ECG suggesting ischemia or recent myocardial infarction.
- Unstable angina
- Uncontrolled cardiac dysrhythmias
- Uncontrolled heart failure
- Severe aortic stenosis
- Pulmonary embolus or pulmonary infarction
- Heart murmur
- Suspected or known dissecting aneurysm

Metabolic

- Type I Diabetes

Other

- Mental or physical impairment leading to inability to exercise adequately
- Cigarette smokers

If you believe your patient is suitable for this research project we would be pleased for you to recommend their participation.

I, _____, a Medical Practitioner registered in the State of Queensland, have reviewed **Subject Name**'s screening information provided by the research team. In conjunction with my own records and the NH&MRC Project Exclusion Criteria provided in the box above

I do recommend / I do not recommend **(please circle one) Subject Name** participate in the exercise program as outlined in the Information Sheet.

In giving my recommendation that **Subject Name** participate in this research project, I acknowledge that I am not liable in anyway for any adverse event or injury that may be sustained during or resulting from the project. I am providing my medical opinion of her suitability to participate in this research as outlined in the Information Sheet.

Doctor's full name (printed): _____

Provider Number: _____

Doctor's signature: _____ Date: _____

Surgery/Practice Contact details:

Phone: _____ Fax: _____

Address: _____

APPENDIX E

Blood Sampling Methodology

All blood samples were collected by an accredited phlebotomist. Subjects were asked to refrain from moderate-to-vigorous physical activity and the consumption of alcohol of caffeine for at least 24 h prior to blood collection. All samples were collected following an overnight (~12 h) fast to ensure food and fluid intake was standardised. All blood samples were collected within 90 s of tourniquet application using a 21 gauge needle. EDTA was used as an anticoagulant and all analyses were completed within 3 h of blood collection. Methodology and instrumentation used for each dependent variable is listed below.

Measure	**Instrument**	**Methodology**
Insulin	Abbott Architect®	Chemiluminescent microparticle immunoassay
Homocysteine	Abbott Architect®	Chemiluminescent microparticle immunoassay
Glucose	Roche Cobas® Modular	Enzymatic (hexokinase)
HbA_{1c}	Bio-rad Variant™ II	High-performance liquid chromatography
Cholesterol	Roche Cobas® Modular	Enzymatic (esterase and cholesterol oxidase)
Triglycerides	Roche Cobas® Modular	Enzymatic (hexokinase)
High-density Lipoprotein	Roche Cobas® Modular	Homogeneous PEG-treated enzymatic
Low-density Lipoprotein	Roche Cobas® Modular	Calculated or Enzymatic (if Trig>4.5 mmol/L)
Red Blood Cell	Sysmex® XT-2000i	Hydro Dynamic Focusing
White Blood Cell	Sysmex® XT-2000i	Flow Cytometry
Haemoglobin	Sysmex® XT-2000i	Sodium Lauryl Sulphate haemoglobin detection